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- (71) Applicant (*for all designated States except US*): **ABBOTT LABORATORIES (BERMUDA) LTD.** [GB/GB]; Clarendon House, 2 Church Street, HM 11 Hamilton (BM).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **KEMPENI, Joachim** [DE/DE]; 75 Gimmeldinger Street, 67433 Neustadt (DE). **WEISS, Roberta** [US/US]; 936 Clover Hill Road, Wynnewood, PA 19096 (US). **FISCHKOFF, Steven, A.** [US/US]; 5 Canoe Brook Road, Short Hills, NJ 07078 (US).
- (74) Agents: **CONWAY, John, D.**; Abbott Bioresearch Center, 100 Research Drive, Worcester, MA 01605-4314 et al. (US).

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**WO 02/100330 A2**

(54) Title: METHODS OF ADMINISTERING ANTI-TNF $\alpha$  ANTIBODIES

(57) **Abstract:** Methods of treating disorders in which TNF $\alpha$  activity is detrimental via biweekly, subcutaneous administration of human antibodies, preferably recombinant human antibodies, that specifically bind to human tumor necrosis factor  $\alpha$  (hTNF $\alpha$ ) are disclosed. The antibody may be administered with or without methotrexate. These antibodies have high affinity for hTNF $\alpha$  (e.g.,  $K_d = 10^{-8}$  M or less), a slow off rate for hTNF $\alpha$  dissociation (e.g.,  $K_{off} = 10^{-3}$  sec $^{-1}$  or less) and neutralize hTNF $\alpha$  activity *in vitro* and *in vivo*. An antibody of the invention can be a full-length antibody or an antigen-binding portion thereof. Kits containing a pharmaceutical composition and instructions for dosing, and preloaded syringes containing pharmaceutical compositions are also encompassed by the invention.

## METHODS OF ADMINISTERING ANTI-TNF $\alpha$ ANTIBODIES

### Background of the Invention

Tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) is a cytokine produced by numerous cell types, including monocytes and macrophages, that was originally identified based on its capacity to induce the necrosis of certain mouse tumors (see e.g., Old, L. (1985) *Science* 230:630-632). Subsequently, a factor termed cachectin, associated with cachexia, was shown to be the same molecule as TNF $\alpha$ . TNF $\alpha$  has been implicated in mediating shock (see e.g., Beutler, B. and Cerami, A. (1988) *Annu. Rev. Biochem.* 57:505-518; Beutler, B. and Cerami, A. (1989) *Annu. Rev. Immunol.* 7:625-655). Furthermore, TNF $\alpha$  has been implicated in the pathophysiology of a variety of other human diseases and disorders, including sepsis, infections, autoimmune diseases, transplant rejection and graft-versus-host disease (see e.g., Vasilli, P. (1992) *Annu. Rev. Immunol.* 10:411-452; Tracey, K.J. and Cerami, A. (1994) *Annu. Rev. Med.* 45:491-503).

Because of the harmful role of human TNF $\alpha$  (hTNF $\alpha$ ) in a variety of human disorders, therapeutic strategies have been designed to inhibit or counteract hTNF $\alpha$  activity. In particular, antibodies that bind to, and neutralize, hTNF $\alpha$  have been sought as a means to inhibit hTNF $\alpha$  activity. Some of the earliest of such antibodies were mouse monoclonal antibodies (mAbs), secreted by hybridomas prepared from lymphocytes of mice immunized with hTNF $\alpha$  (see e.g., Hahn T; et al., (1985) *Proc Natl Acad Sci USA* 82: 3814-3818; Liang, C.-M., et al. (1986) *Biochem. Biophys. Res. Commun.* 137:847-854; Hirai, M., et al. (1987) *J. Immunol. Methods* 96:57-62; Fendly, B.M., et al. (1987) *Hybridoma* 6:359-370; Möller, A., et al. (1990) *Cytokine* 2:162-169; U.S. Patent No. 5,231,024 to Moeller et al.; European Patent Publication No. 186 833 B1 by Wallach, D.; European Patent Application Publication No. 218 868 A1 by Old et al.; European Patent Publication No. 260 610 B1 by Moeller, A., et al.). While these mouse anti-hTNF $\alpha$  antibodies often displayed high affinity for hTNF $\alpha$  (e.g.,  $K_d \leq 10^{-9} M$ ) and were able to neutralize hTNF $\alpha$  activity, their use *in vivo* may be limited by problems associated with administration of mouse antibodies to humans, such as short serum half life, an inability to trigger certain human effector functions and elicitation of an unwanted immune response against the mouse antibody in a human (the "human anti-mouse antibody" (HAMA) reaction).

In an attempt to overcome the problems associated with use of fully-murine antibodies in humans, murine anti-hTNF $\alpha$  antibodies have been genetically engineered to be more "human-like." For example, chimeric antibodies, in which the variable regions of the antibody chains are murine-derived and the constant regions of the antibody chains are human-derived, have been prepared (Knight, D.M, et al. (1993) *Mol. Immunol.* 30:1443-1453; PCT Publication No. WO 92/16553 by Daddona, P.E., et al.). Additionally, humanized antibodies, in which the hypervariable domains of the antibody variable regions are murine-derived but the remainder of the variable regions and the antibody constant

regions are human-derived, have also been prepared (PCT Publication No. WO 92/11383 by Adair, J.R., *et al.*). However, because these chimeric and humanized antibodies still retain some murine sequences, they still may elicit an unwanted immune reaction, the human anti-chimeric antibody (HACA) reaction, especially when administered for prolonged periods, 5 e.g., for chronic indications, such as rheumatoid arthritis (see e.g., Elliott, M.J., *et al.* (1994) *Lancet* 344:1125-1127; Elliot, M.J., *et al.* (1994) *Lancet* 344:1105-1110).

A preferred hTNF $\alpha$  inhibitory agent to murine mAbs or derivatives thereof (e.g., chimeric or humanized antibodies) would be an entirely human anti-hTNF $\alpha$  antibody, since such an agent should not elicit the HAMA reaction, even if used for prolonged periods.

10 Human monoclonal autoantibodies against hTNF $\alpha$  have been prepared using human hybridoma techniques (Boyle, P., *et al.* (1993) *Cell. Immunol.* 152:556-568; Boyle, P., *et al.* (1993) *Cell. Immunol.* 152:569-581; European Patent Application Publication No. 614 984 A2 by Boyle, *et al.*). However, these hybridoma-derived monoclonal autoantibodies were reported to have an affinity for hTNF $\alpha$  that was too low to calculate by conventional 15 methods, were unable to bind soluble hTNF $\alpha$  and were unable to neutralize hTNF $\alpha$ -induced cytotoxicity (see Boyle, *et al.*; *supra*). Moreover, the success of the human hybridoma technique depends upon the natural presence in human peripheral blood of lymphocytes producing autoantibodies specific for hTNF $\alpha$ . Certain studies have detected serum autoantibodies against hTNF $\alpha$  in human subjects (Fomsgaard, A., *et al.* (1989) *Scand. J. 20 Immunol.* 30:219-223; Bendtzen, K., *et al.* (1990) *Prog. Leukocyte Biol.* 10B:447-452), whereas others have not (Leusch, H-G., *et al.* (1991) *J. Immunol. Methods* 139:145-147).

Alternative to naturally-occurring human anti-hTNF $\alpha$  antibodies would be a recombinant hTNF $\alpha$  antibody. Recombinant human antibodies that bind hTNF $\alpha$  with relatively low affinity (*i.e.*,  $K_d \sim 10^{-7}$  M) and a fast off rate (*i.e.*,  $K_{off} \sim 10^{-2}$  sec $^{-1}$ ) have been 25 described (Griffiths, A.D., *et al.* (1993) *EMBO J.* 12:725-734). However, because of their relatively fast dissociation kinetics, these antibodies may not be suitable for therapeutic use. Additionally, a recombinant human anti-hTNF $\alpha$  has been described that does not neutralize hTNF $\alpha$  activity, but rather enhances binding of hTNF $\alpha$  to the surface of cells and enhances internalization of hTNF $\alpha$  (Lidbury, A., *et al.* (1994) *Biotechnol. Ther.* 5:27-45; PCT 30 Publication No. WO 92/03145 by Aston, R. *et al.*)

Recombinant human antibodies that bind soluble hTNF $\alpha$  with high affinity and slow dissociation kinetics and that have the capacity to neutralize hTNF $\alpha$  activity, including hTNF $\alpha$ -induced cytotoxicity (*in vitro* and *in vivo*) and hTNF $\alpha$ -induced cell activation, have 35 also been described (see U.S. Patent No. 6,090,382). Typical protocols for administering antibodies are performed intravenously on a weekly basis. Weekly dosing with antibodies and/or any drug can be costly, cumbersome, and result in an increase in the number of side effects due to the frequency of administration. Intravenous administration also has limitations in that the administration is usually provided by someone with medical training.

Summary of the Invention

The present invention provides methods for biweekly dosing regimens for the treatment of TNF $\alpha$  associated disorders, preferably via a subcutaneous route. Biweekly dosing has many advantages over weekly dosing including, but not limited to, a lower number of total injections, decreased number of injection site reactions (e.g., local pain and swelling), increased patient compliance (i.e., due to less frequent injections), and less cost to the patient as well as the health care provider. Subcutaneous dosing is advantageous because the patient may self-administer a therapeutic substance, e.g., a human TNF $\alpha$  antibody, which is convenient for both the patient and the health care provider.

This invention provides methods for treating disorders in which TNF $\alpha$  activity is detrimental. The methods include administering biweekly, subcutaneous injections of antibodies to a subject. The antibodies preferably are recombinant human antibodies that specifically bind to human TNF $\alpha$ . This invention further provides methods for treating disorders in which TNF $\alpha$  activity is detrimental. These methods include utilizing a combination therapy wherein human antibodies are administered to a subject with another therapeutic agent, such as one or more additional antibodies that bind other targets (e.g., antibodies that bind other cytokines or that bind cell surface molecules), one or more cytokines, soluble TNF $\alpha$  receptor (see e.g., PCT Publication No. WO 94/06476) and/or one or more chemical agents that inhibit hTNF $\alpha$  production or activity (such as cyclohexane-ylidene derivatives as described in PCT Publication No. WO 93/19751), preferably methotrexate. The antibodies are preferably recombinant human antibodies that specifically bind to human TNF $\alpha$ . The antibodies of the invention are characterized by binding to hTNF $\alpha$  with high affinity and slow dissociation kinetics and by neutralizing hTNF $\alpha$  activity, including hTNF $\alpha$ -induced cytotoxicity (*in vitro* and *in vivo*) and hTNF $\alpha$ -induced cellular activation. The antibodies can be full-length (e.g., an IgG1 or IgG4 antibody) or can comprise only an antigen-binding portion (e.g., a Fab, F(ab')<sub>2</sub>, scFv fragment or single domain). The most preferred recombinant antibody of the invention, termed D2E7, has a light chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3 and a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4 (set forth in Appendix B). Preferably, the D2E7 antibody has a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 1 and a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 2. These antibodies are described in U.S. Patent No. 6,090,382, incorporated in its entirety herein by reference.

In one embodiment, the invention provides methods of treating disorders in which TNF $\alpha$  activity is detrimental. These methods include inhibiting human TNF $\alpha$  activity by subcutaneous, biweekly administration of an anti-TNF $\alpha$  antibody such that the disorder is treated. The disorder can be, for example, sepsis, an autoimmune disease (e.g., rheumatoid arthritis, allergy, multiple sclerosis, autoimmune diabetes, autoimmune uveitis and nephrotic

syndrome), an infectious disease, a malignancy, transplant rejection or graft-versus-host disease, a pulmonary disorder, a bone disorder, an intestinal disorder or a cardiac disorder.

In another embodiment, the invention provides methods of treating disorders in which TNF $\alpha$  activity is detrimental. These methods include inhibiting human TNF $\alpha$  activity by 5 subcutaneous administration of an anti-TNF $\alpha$  antibody and methotrexate such that the disorder is treated. In one aspect, methotrexate is administered together with an anti-TNF $\alpha$  antibody. In another aspect, methotrexate is administered prior to the administration of an anti-TNF $\alpha$  antibody. In still another aspect, methotrexate is administered subsequent to the administration of an anti-TNF $\alpha$  antibody.

10 In a preferred embodiment, the anti-TNF $\alpha$  antibody used to treat disorders in which TNF $\alpha$  activity is detrimental is a human anti-TNF $\alpha$  antibody. Even more preferably, treatment occurs by the biweekly, subcutaneous administration of an isolated human antibody, or an antigen-binding portion thereof. The antibody or antigen-binding portion thereof preferably dissociates from human TNF $\alpha$  with a  $K_d$  of  $1 \times 10^{-8}$  M or less and a  $K_{off}$  15 rate constant of  $1 \times 10^{-3} s^{-1}$  or less, both determined by surface plasmon resonance, and neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC<sub>50</sub> of  $1 \times 10^{-7}$  M or less. More preferably, the isolated human antibody, or antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  of  $5 \times 10^{-4} s^{-1}$  or less, or even more 20 preferably, with a  $K_{off}$  of  $1 \times 10^{-4} s^{-1}$  or less. More preferably, the isolated human antibody, or antigen-binding portion thereof, neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC<sub>50</sub> of  $1 \times 10^{-8}$  M or less, even more preferably with an IC<sub>50</sub> of  $1 \times 10^{-9}$  M or less and still more preferably with an IC<sub>50</sub> of  $1 \times 10^{-10}$  M or less.

25 In another embodiment, the invention provides methods of treating disorders in which TNF $\alpha$  activity is detrimental by the biweekly, subcutaneous administration to the subject a human antibody, or antigen-binding portion thereof. The antibody or antigen-binding portion thereof preferably has the following characteristics:

- a) dissociates from human TNF $\alpha$  with a  $K_{off}$  of  $1 \times 10^{-3} s^{-1}$  or less, as determined by surface plasmon resonance;
- b) has a light chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8 or by one to five conservative amino acid substitutions at positions 1, 3, 4, 6, 7, 8 and/or 9;
- c) has a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 or by one to five conservative amino acid substitutions at positions 2, 3, 4, 5, 6, 8, 9, 10, 11 and/or 12.

More preferably, the antibody, or antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  of  $5 \times 10^{-4} s^{-1}$  or less. Still more preferably, the antibody, or

antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  of  $1 \times 10^{-4} \text{ s}^{-1}$  or less.

In yet another embodiment, the invention provides methods of treating disorders in which TNF $\alpha$  activity is detrimental. These methods include a biweekly, subcutaneous administration to the subject a human antibody, or an antigen-binding portion thereof. The antibody or antigen-binding portion thereof preferably contains an LCVR having CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8, and with an HCVR having a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11. More preferably, the LCVR further has a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 5 and the HCVR further has a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 6. Still more preferably, the LCVR further has CDR1 domain comprising the amino acid sequence of SEQ ID NO: 7 and the HCVR has a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 8.

In still another embodiment, the invention provides methods of treating disorders in which TNF $\alpha$  activity is detrimental by subcutaneously administering to the subject, biweekly, an isolated human antibody, or an antigen binding portion thereof. The antibody or antigen-binding portion thereof preferably contains an LCVR comprising the amino acid sequence of SEQ ID NO: 1 and an HCVR comprising the amino acid sequence of SEQ ID NO: 2. In certain embodiments, the antibody has an IgG1 heavy chain constant region or an IgG4 heavy chain constant region. In yet other embodiments, the antibody is a Fab fragment, an F(ab')<sub>2</sub> fragment or a single chain Fv fragment.

In still other embodiments, the invention provides methods of treating disorders in which the administration of an anti-TNF $\alpha$  antibody is beneficial by subcutaneously administering to the subject, biweekly, one or more anti-TNF $\alpha$  antibodies, or antigen-binding portions thereof. The antibody or antigen-binding portion thereof preferably contains an LCVR having CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26 or with an HCVR having a CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34 and SEQ ID NO: 35.

Still another aspect of the invention pertains to kits containing a formulation comprising a pharmaceutical composition. The kits comprise an anti-TNF $\alpha$  antibody and a pharmaceutically acceptable carrier. The kits contain instructions for biweekly subcutaneous dosing of the pharmaceutical composition for the treatment of a disorder in which the

administration of an anti-TNF $\alpha$  antibody is beneficial. In another aspect, the invention pertains to kits containing a formulation comprising a pharmaceutical composition, further comprising an anti-TNF $\alpha$  antibody, methotrexate, and a pharmaceutically acceptable carrier. The kits contain instructions for subcutaneous dosing of the pharmaceutical composition for 5 the treatment of a disorder in which the administration of an anti-TNF $\alpha$  antibody is beneficial.

Still another aspect of the invention provides a preloaded syringe containing a pharmaceutical composition comprising an anti-TNF $\alpha$  antibody and a pharmaceutically acceptable carrier. In still another aspect, the invention provides a preloaded syringe 10 containing a pharmaceutical composition comprising an anti-TNF $\alpha$  antibody, methotrexate, and a pharmaceutically acceptable carrier.

#### Brief Description of the Drawings

Figures 1A and 1B depict the American College of Rheumatology 20 (ACR20) and 15 ACR50 responses for patients suffering from rheumatoid arthritis (RA) after subcutaneous dosing with the antibody D2E7 every week for a total of twelve weeks (1A), or subcutaneous dosing with the antibody D2E7 and methotrexate every other week (1B) for a total of twenty-four weeks. These data indicate that every other week dosing is as effective as every week dosing.

Figure 2 depicts ACR20, ACR50, and ACR70 responses for patients suffering from 20 RA after subcutaneous dosing with the antibody D2E7 and methotrexate every other week at twenty-four weeks.

Figures 3A and 3B depict time courses of tender joint count (3A) and swollen joint 25 count (3B) over twenty-four weeks for patients suffering from RA after subcutaneous dosing with D2E7 and methotrexate every other week at twenty-four weeks.

Figure 4 depicts results from a short form health survey (SF-36) from patients suffering from RA after subcutaneous dosing with the antibody D2E7 and methotrexate every other week at twenty-four weeks. RP, role physical; PF, physical function; BP, bodily pain; GH, general health; V, vitality; SF, social functioning; RE, role emotional; and ME, mental 30 health.

Figure 5 depicts the percentage of ACR responders following a single intravenous injection of the antibody D2E7 and methotrexate in patients suffering from RA.

#### Detailed Description of the Invention

This invention pertains to methods of treating disorders in which the administration of 35 an anti-TNF $\alpha$  antibody is beneficial comprising the administration of isolated human antibodies, or antigen-binding portions thereof, that bind to human TNF $\alpha$  with high affinity, a low off rate and high neutralizing capacity such that the disorder is treated. Various aspects

of the invention relate to treatment with antibodies and antibody fragments, and pharmaceutical compositions thereof.

In order that the present invention may be more readily understood, certain terms are first defined.

5        The term "dosing", as used herein, refers to the administration of a substance (e.g., an anti-TNF $\alpha$  antibody) to achieve a therapeutic objective (e.g., the treatment of a TNF $\alpha$ -associated disorder).

10      The terms "biweekly dosing regimen", "biweekly dosing", and "biweekly administration", as used herein, refer to the time course of administering a substance (e.g., an anti-TNF $\alpha$  antibody) to a subject to achieve a therapeutic objective (e.g., the treatment of a TNF $\alpha$ -associated disorder). The biweekly dosing regimen is not intended to include a weekly dosing regimen. Preferably, the substance is administered every 9-19 days, more preferably, every 11-17 days, even more preferably, every 13-15 days, and most preferably, every 14 days.

15      The term "combination therapy", as used herein, refers to the administration of two or more therapeutic substances, e.g., an anti-TNF $\alpha$  antibody and the drug methotrexate. The methotrexate may be administered concomitant with, prior to, or following the administration of an anti-TNF $\alpha$  antibody.

20      The term "human TNF $\alpha$ " (abbreviated herein as hTNF $\alpha$ , or simply hTNF), as used herein, is intended to refer to a human cytokine that exists as a 17 kD secreted form and a 26 kD membrane associated form, the biologically active form of which is composed of a trimer of noncovalently bound 17 kD molecules. The structure of TNF $\alpha$  is described further in, for example, Pennica, D., *et al.* (1984) *Nature* 312:724-729; Davis, J.M., *et al.* (1987) *Biochemistry* 26:1322-1326; and Jones, E.Y., *et al.* (1989) *Nature* 338:225-228. The term 25 human TNF $\alpha$  is intended to include recombinant human TNF $\alpha$  (rhTNF $\alpha$ ), which can be prepared by standard recombinant expression methods or purchased commercially (R & D Systems, Catalog No. 210-TA, Minneapolis, MN).

30      The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. 35 The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., hTNF $\alpha$ ). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples 5 of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')<sub>2</sub> fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an 10 antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent 15 molecules (known as single chain Fv (scFv); see e.g., Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on 20 a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see e.g., Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123).

25 Still further, an antibody or antigen-binding portion thereof may be part of a larger immunoadhesion molecules, formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov, S.M., *et al.* (1995) *Human Antibodies and Hybridomas* 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov, S.M., *et al.* (1994) *Mol. Immunol.* 31:1047-1058). Antibody portions, such as Fab and F(ab')<sub>2</sub> fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and 30 immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein.

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human

germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another 5 mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host 10 cell (described further in Section II, below), antibodies isolated from a recombinant, combinatorial human antibody library (described further in Section III, below), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor, L.D., *et al.* (1992) *Nucl. Acids Res.* **20**:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human 15 immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL 20 regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds hTNF $\alpha$  is substantially free of antibodies that specifically 25 bind antigens other than hTNF $\alpha$ ). An isolated antibody that specifically binds hTNF $\alpha$  may, however, have cross-reactivity to other antigens, such as hTNF $\alpha$  molecules from other species (discussed in further detail below). Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

A "neutralizing antibody", as used herein (or an "antibody that neutralized hTNF $\alpha$  30 activity"), is intended to refer to an antibody whose binding to hTNF $\alpha$  results in inhibition of the biological activity of hTNF $\alpha$ . This inhibition of the biological activity of hTNF $\alpha$  can be assessed by measuring one or more indicators of hTNF $\alpha$  biological activity, such as hTNF $\alpha$ -induced cytotoxicity (either *in vitro* or *in vivo*), hTNF $\alpha$ -induced cellular activation and hTNF $\alpha$  binding to hTNF $\alpha$  receptors. These indicators of hTNF $\alpha$  biological activity can be 35 assessed by one or more of several standard *in vitro* or *in vivo* assays known in the art (see Example 4). Preferably, the ability of an antibody to neutralize hTNF $\alpha$  activity is assessed by inhibition of hTNF $\alpha$ -induced cytotoxicity of L929 cells. As an additional or alternative parameter of hTNF $\alpha$  activity, the ability of an antibody to inhibit hTNF $\alpha$ -induced expression of ELAM-1 on HUVEC, as a measure of hTNF $\alpha$ -induced cellular activation, can be assessed.

The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Example 1 and Jönsson, U., et al. (1993) *Ann. Biol. Clin.* 51:19-26; Jönsson, U., et al. (1991) *Biotechniques* 11:620-627; Johnsson, B., et al. (1995) *J. Mol. Recognit.* 8:125-131; and Johnnson, B., et al. (1991) *Anal. Biochem.* 198:268-277.

The term " $K_{off}$ ", as used herein, is intended to refer to the off rate constant for dissociation of an antibody from the antibody/antigen complex.

10 The term " $K_d$ ", as used herein, is intended to refer to the dissociation constant of a particular antibody-antigen interaction.

The term "nucleic acid molecule", as used herein, is intended to include DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA.

15 The term "isolated nucleic acid molecule", as used herein in reference to nucleic acids encoding antibodies or antibody portions (e.g., VH, VL, CDR3) that bind hTNF $\alpha$ , is intended to refer to a nucleic acid molecule in which the nucleotide sequences encoding the antibody or antibody portion are free of other nucleotide sequences encoding antibodies or antibody portions that bind antigens other than hTNF $\alpha$ , which other sequences may naturally flank the 20 nucleic acid in human genomic DNA. Thus, for example, an isolated nucleic acid of the invention encoding a VH region of an anti-hTNF $\alpha$  antibody contains no other sequences encoding other VH regions that bind antigens other than hTNF $\alpha$ .

The term "vector", as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is 25 a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant 30 DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding 5 generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

Various aspects of the invention are described in further detail in the following subsections.

10

### I. Human Antibodies that Bind Human TNF $\alpha$

This invention provides methods of treating disorders in which the administration of an anti-TNF $\alpha$  antibody is beneficial. These methods include the biweekly, subcutaneous administration of isolated human antibodies, or antigen-binding portions thereof, that bind to 15 human TNF $\alpha$  with high affinity, a low off rate and high neutralizing capacity. Preferably, the human antibodies of the invention are recombinant, neutralizing human anti-hTNF $\alpha$  antibodies. The most preferred recombinant, neutralizing antibody of the invention is referred to herein as D2E7 (the amino acid sequence of the D2E7 VL region is shown in SEQ ID NO: 1; the amino acid sequence of the D2E7 VH region is shown in SEQ ID NO: 2). The 20 properties of D2E7 have been described in Salfeld *et al.*, U.S. patent No. 6,090,382, which is incorporated by reference herein.

In one aspect, the invention pertains to treating disorders in which the administration of an anti-TNF $\alpha$  antibody is beneficial. These treatments include the biweekly, subcutaneous administration of D2E7 antibodies and antibody portions, D2E7-related antibodies and 25 antibody portions, and other human antibodies and antibody portions with equivalent properties to D2E7, such as high affinity binding to hTNF $\alpha$  with low dissociation kinetics and high neutralizing capacity. In one embodiment, the invention provides treatment with an isolated human antibody, or an antigen-binding portion thereof, that dissociates from human TNF $\alpha$  with a  $K_d$  of  $1 \times 10^{-8}$  M or less and a  $K_{off}$  rate constant of  $1 \times 10^{-3} \text{ s}^{-1}$  or less, both 30 determined by surface plasmon resonance, and neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC<sub>50</sub> of  $1 \times 10^{-7}$  M or less. More preferably, the isolated human antibody, or antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  of  $5 \times 10^{-4} \text{ s}^{-1}$  or less, or even more preferably, with a  $K_{off}$  of  $1 \times 10^{-4} \text{ s}^{-1}$  or less. More preferably, the isolated human antibody, or antigen-binding portion thereof, 35 neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC<sub>50</sub> of  $1 \times 10^{-8}$  M or less, even more preferably with an IC<sub>50</sub> of  $1 \times 10^{-9}$  M or less and still more preferably with an IC<sub>50</sub> of  $1 \times 10^{-10}$  M or less. In a preferred embodiment, the antibody is an isolated human recombinant antibody, or an antigen-binding portion thereof.

It is well known in the art that antibody heavy and light chain CDR3 domains play an important role in the binding specificity/affinity of an antibody for an antigen. Accordingly, in another aspect, the invention pertains to methods of treating disorders in which the administration of an anti-TNF $\alpha$  antibody is beneficial by subcutaneous administration of

5 human antibodies that have slow dissociation kinetics for association with hTNF $\alpha$  and that have light and heavy chain CDR3 domains that structurally are identical to or related to those of D2E7. Position 9 of the D2E7 VL CDR3 can be occupied by Ala or Thr without substantially affecting the K<sub>off</sub>. Accordingly, a consensus motif for the D2E7 VL CDR3 comprises the amino acid sequence: Q-R-Y-N-R-A-P-Y-(T/A) (SEQ ID NO: 3).

10 Additionally, position 12 of the D2E7 VH CDR3 can be occupied by Tyr or Asn, without substantially affecting the K<sub>off</sub>. Accordingly, a consensus motif for the D2E7 VH CDR3 comprises the amino acid sequence: V-S-Y-L-S-T-A-S-S-L-D-(Y/N) (SEQ ID NO: 4). Moreover, as demonstrated in Example 2, the CDR3 domain of the D2E7 heavy and light chains is amenable to substitution with a single alanine residue (at position 1, 4, 5, 7 or 8

15 within the VL CDR3 or at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 within the VH CDR3) without substantially affecting the K<sub>off</sub>. Still further, the skilled artisan will appreciate that, given the amenability of the D2E7 VL and VH CDR3 domains to substitutions by alanine, substitution of other amino acids within the CDR3 domains may be possible while still retaining the low off rate constant of the antibody, in particular substitutions with conservative amino acids. A

20 "conservative amino acid substitution", as used herein, is one in which one amino acid residue is replaced with another amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine,

25 tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Preferably, no more than one to five conservative amino acid substitutions are made within the D2E7 VL and/or VH CDR3 domains. More preferably, no more than one to three

30 conservative amino acid substitutions are made within the D2E7 VL and/or VH CDR3 domains. Additionally, conservative amino acid substitutions should not be made at amino acid positions critical for binding to hTNF $\alpha$ . Positions 2 and 5 of the D2E7 VL CDR3 and positions 1 and 7 of the D2E7 VH CDR3 appear to be critical for interaction with hTNF $\alpha$  and thus, conservative amino acid substitutions preferably are not made at these positions

35 (although an alanine substitution at position 5 of the D2E7 VL CDR3 is acceptable, as described above) (see U.S. Patent No. 6,090,382).

Accordingly, in another embodiment, the invention provides methods of treating disorders in which the administration of an anti-TNF $\alpha$  antibody is beneficial by the biweekly, subcutaneous administration of an isolated human antibody, or antigen-binding portion

thereof. The antibody or antigen-binding portion thereof preferably contains the following characteristics:

a) dissociates from human TNF $\alpha$  with a  $K_{off}$  rate constant of  $1 \times 10^{-3} \text{ s}^{-1}$  or less, as determined by surface plasmon resonance;

5 b) has a light chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8 or by one to five conservative amino acid substitutions at positions 1, 3, 4, 6, 7, 8 and/or 9;

c) has a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 or by one to five conservative amino acid substitutions at positions 2, 3, 4, 5, 6, 8, 9, 10, 11 and/or 12.

10 More preferably, the antibody, or antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  of  $5 \times 10^{-4} \text{ s}^{-1}$  or less. Even more preferably, the antibody, or 15 antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  of  $1 \times 10^{-4} \text{ s}^{-1}$  or less.

In yet another embodiment, the invention provides methods of treating disorders in which the administration of an anti-TNF $\alpha$  antibody is beneficial by the biweekly, subcutaneous administration of an isolated human antibody, or an antigen-binding portion 20 thereof. The antibody or antigen-binding portion thereof preferably contains a light chain variable region (LCVR) having a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8, and with a heavy chain variable region (HCVR) having a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single 25 alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11. Preferably, the LCVR further has a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 5 (*i.e.*, the D2E7 VL CDR2) and the HCVR further has a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 6 (*i.e.*, the D2E7 VH CDR2). Even more preferably, the LCVR further has CDR1 domain comprising the amino acid sequence of SEQ ID NO: 7 (*i.e.*, the D2E7 VL 30 CDR1) and the HCVR has a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 8 (*i.e.*, the D2E7 VH CDR1). The framework regions for VL preferably are from the V<sub>KI</sub> human germline family, more preferably from the A20 human germline V<sub>K</sub> gene and most preferably from the D2E7 VL framework sequences shown in Figures 1A and 1B of U.S. Patent No. 6,090,382. The framework regions for VH preferably are from the V<sub>H3</sub> 35 human germline family, more preferably from the DP-31 human germline VH gene and most preferably from the D2E7 VH framework sequences shown in Figures 2A and 2B U.S. Patent No. 6,090,382.

In still another embodiment, the invention provides methods of treating disorders in which the administration of an anti-TNF $\alpha$  antibody is beneficial by the biweekly,

subcutaneous administration of an isolated human antibody, or an antigen binding portion thereof. The antibody or antigen-binding portion thereof preferably contains a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 1 (*i.e.*, the D2E7 VL) and a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 2 (*i.e.*, the D2E7 VH). In certain embodiments, the antibody comprises a heavy chain constant region, such as an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region. Preferably, the heavy chain constant region is an IgG1 heavy chain constant region or an IgG4 heavy chain constant region. Furthermore, the antibody can comprise a light chain constant region, either a kappa light chain constant region or a lambda light chain constant region. Preferably, the antibody comprises a kappa light chain constant region. Alternatively, the antibody portion can be, for example, a Fab fragment or a single chain Fv fragment.

In still other embodiments, the invention provides methods of treating disorders in which the administration of an anti-TNF $\alpha$  antibody is beneficial by the biweekly, subcutaneous administration of an isolated human antibody, or an antigen-binding portions thereof. The antibody or antigen-binding portion thereof preferably contains D2E7-related VL and VH CDR3 domains, for example, antibodies, or antigen-binding portions thereof, with a light chain variable region (LCVR) having a CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26 or with a heavy chain variable region (HCVR) having a CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34 and SEQ ID NO: 35.

An antibody or antibody portion of the invention can be derivatized or linked to another functional molecule (*e.g.*, another peptide or protein). Accordingly, the antibodies and antibody portions of the invention are intended to include derivatized and otherwise modified forms of the human anti-hTNF $\alpha$  antibodies described herein, including immunoadhesion molecules. For example, an antibody or antibody portion of the invention can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (*e.g.*, a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate associate of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

One type of derivatized antibody is produced by crosslinking two or more antibodies (of the same type or of different types, *e.g.*, to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups

separated by an appropriate spacer (*e.g.*, m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (*e.g.*, disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, IL.

Useful detectable agents with which an antibody or antibody portion of the invention 5 may be derivatized include fluorescent compounds. Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin and the like. An antibody may also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, glucose 10 oxidase and the like. When an antibody is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example, when the detectable agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody may also be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding.

15

## II. Expression of Antibodies

An antibody, or antibody portion, of the invention can be prepared by recombinant expression of immunoglobulin light and heavy chain genes in a host cell. To express an antibody recombinantly, a host cell is transfected with one or more recombinant expression 20 vectors carrying DNA fragments encoding the immunoglobulin light and heavy chains of the antibody such that the light and heavy chains are expressed in the host cell and, preferably, secreted into the medium in which the host cells are cultured, from which medium the antibodies can be recovered. Standard recombinant DNA methodologies are used to obtain antibody heavy and light chain genes, incorporate these genes into recombinant expression 25 vectors and introduce the vectors into host cells, such as those described in Sambrook, Fritsch and Maniatis (eds), *Molecular Cloning; A Laboratory Manual, Second Edition*, Cold Spring Harbor, N.Y., (1989), Ausubel, F.M. *et al.* (eds.) *Current Protocols in Molecular Biology*, Greene Publishing Associates, (1989) and in U.S. Patent No. 4,816,397 by Boss *et al.*

To express D2E7 or a D2E7-related antibody, DNA fragments encoding the light and 30 heavy chain variable regions are first obtained. These DNAs can be obtained by amplification and modification of germline light and heavy chain variable sequences using the polymerase chain reaction (PCR). Germline DNA sequences for human heavy and light chain variable region genes are known in the art (*see e.g.*, the "Vbase" human germline sequence database; *see also* Kabat, E.A., *et al.* (1991) *Sequences of Proteins of 35 Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Tomlinson, I.M., *et al.* (1992) "The Repertoire of Human Germline V<sub>H</sub> Sequences Reveals about Fifty Groups of V<sub>H</sub> Segments with Different Hypervariable Loops" *J. Mol. Biol.* 227:776-798; and Cox, J.P.L. *et al.* (1994) "A Directory of Human Germ-line V<sub>γ8</sub> Segments Reveals a Strong Bias in their Usage" *Eur. J. Immunol.* 24:827-

836; the contents of each of which are expressly incorporated herein by reference). To obtain a DNA fragment encoding the heavy chain variable region of D2E7, or a D2E7-related antibody, a member of the V<sub>H</sub>3 family of human germline VH genes is amplified by standard PCR. Most preferably, the DP-31 VH germline sequence is amplified. To obtain a DNA 5 fragment encoding the light chain variable region of D2E7, or a D2E7-related antibody, a member of the V<sub>K</sub>I family of human germline VL genes is amplified by standard PCR. Most preferably, the A20 VL germline sequence is amplified. PCR primers suitable for use in amplifying the DP-31 germline VH and A20 germline VL sequences can be designed based on the nucleotide sequences disclosed in the references cited *supra*, using standard methods.

10 Once the germline VH and VL fragments are obtained, these sequences can be mutated to encode the D2E7 or D2E7-related amino acid sequences disclosed herein. The amino acid sequences encoded by the germline VH and VL DNA sequences are first compared to the D2E7 or D2E7-related VH and VL amino acid sequences to identify amino acid residues in the D2E7 or D2E7-related sequence that differ from germline. Then, the 15 appropriate nucleotides of the germline DNA sequences are mutated such that the mutated germline sequence encodes the D2E7 or D2E7-related amino acid sequence, using the genetic code to determine which nucleotide changes should be made. Mutagenesis of the germline sequences is carried out by standard methods, such as PCR-mediated mutagenesis (in which the mutated nucleotides are incorporated into the PCR primers such that the PCR product 20 contains the mutations) or site-directed mutagenesis.

Once DNA fragments encoding D2E7 or D2E7-related VH and VL segments are obtained (by amplification and mutagenesis of germline VH and VL genes, as described above), these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain 25 genes, to Fab fragment genes or to a scFv gene. In these manipulations, a VL- or VH-encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operatively linked", as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

30 The isolated DNA encoding the VH region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (see e.g., Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human 35 Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region, but most preferably is an IgG1 or IgG4 constant region. For a Fab fragment heavy chain gene, the VH-encoding DNA can be

operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

The isolated DNA encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the VL-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (see e.g., Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region, but most preferably is a kappa constant region.

To create a scFv gene, the VH- and VL-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, e.g., encoding the amino acid sequence (Gly<sub>4</sub>-Ser)<sub>3</sub>, such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VL and VH regions joined by the flexible linker (see e.g., Bird *et al.* (1988) *Science* 242:423-426; Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; McCafferty *et al.*, *Nature* (1990) 348:552-554).

To express the antibodies, or antibody portions of the invention, DNAs encoding partial or full-length light and heavy chains, obtained as described above, are inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vector or, more typically, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (e.g., ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). Prior to insertion of the D2E7 or D2E7-related light or heavy chain sequences, the expression vector may already carry antibody constant region sequences. For example, one approach to converting the D2E7 or D2E7-related VH and VL sequences to full-length antibody genes is to insert them into expression vectors already encoding heavy chain constant and light chain constant regions, respectively, such that the VH segment is operatively linked to the CH segment(s) within the vector and the VL segment is operatively linked to the CL segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the

antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (*i.e.*, a signal peptide from a non-immunoglobulin protein).

In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, *etc.* Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (*e.g.*, the adenovirus major late promoter (AdMLP)) and polyoma. For further description of viral regulatory elements, and sequences thereof, see *e.g.*, U.S. Patent No. 5,168,062 by Stinski, U.S. Patent No. 4,510,245 by Bell *et al.* and U.S. Patent No. 4,968,615 by Schaffner *et al.*

In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see *e.g.*, U.S. Patents Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel *et al.*). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr<sup>-</sup> host cells with methotrexate selection/amplification) and the *neo* gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, and most preferably mammalian host cells, is the most preferred because such eukaryotic cells, and in particular mammalian cells, are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. Prokaryotic expression of

antibody genes has been reported to be ineffective for production of high yields of active antibody (Boss, M.A. and Wood, C. R. (1985) *Immunology Today* 6:12-13).

Preferred mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, e.g., as described in R.J. Kaufman and P.A. Sharp (1982) *Mol. Biol.* 159:601-621), NS0 myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Host cells can also be used to produce portions of intact antibodies, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure are within the scope of the present invention. For example, it may be desirable to transfect a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an antibody of this invention. Recombinant DNA technology may also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to hTNF $\alpha$ . The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an antibody of the invention and the other heavy and light chain are specific for an antigen other than hTNF $\alpha$  by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods.

In a preferred system for recombinant expression of an antibody, or antigen-binding portion thereof, of the invention, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr-CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfet the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium.

### III. Selection of Recombinant Human Antibodies

Recombinant human antibodies of the invention in addition to D2E7 or an antigen binding portion thereof, or D2E7-related antibodies disclosed herein can be isolated by screening of a recombinant combinatorial antibody library, preferably a scFv phage display library, prepared using human VL and VH cDNAs prepared from mRNA derived from human lymphocytes. Methodologies for preparing and screening such libraries are known in the art. In addition to commercially available kits for generating phage display libraries (e.g., the Pharmacia *Recombinant Phage Antibody System*, catalog no. 27-9400-01; and the Stratagene *SurfZAP<sup>TM</sup>* phage display kit, catalog no. 240612), examples of methods and reagents particularly amenable for use in generating and screening antibody display libraries can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT Publication No. WO 92/18619; Dower *et al.* PCT Publication No. WO 91/17271; Winter *et al.* PCT Publication No. WO 92/20791; Markland *et al.* PCT Publication No. WO 92/15679; Breitling *et al.* PCT Publication No. WO 93/01288; McCafferty *et al.* PCT Publication No. WO 92/01047; Garrard *et al.* PCT Publication No. WO 92/09690; Fuchs *et al.* (1991) *BioTechnology* 9:1370-1372; Hay *et al.* (1992) *Hum Antibod Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; McCafferty *et al.*, *Nature* (1990) 348:552-554; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J Mol Biol* 226:889-896; Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrard *et al.* (1991) *BioTechnology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc Acid Res* 19:4133-4137; and Barbas *et al.* (1991) *PNAS* 88:7978-7982.

In a preferred embodiment, to isolate human antibodies with high affinity and a low off rate constant for hTNF $\alpha$ , a murine anti-hTNF $\alpha$  antibody having high affinity and a low off rate constant for hTNF $\alpha$  (e.g., MAK 195, the hybridoma for which has deposit number ECACC 87 050801) is first used to select human heavy and light chain sequences having similar binding activity toward hTNF $\alpha$ , using the epitope imprinting methods described in Hoogenboom *et al.*, PCT Publication No. WO 93/06213. The antibody libraries used in this method are preferably scFv libraries prepared and screened as described in McCafferty *et al.*, PCT Publication No. WO 92/01047, McCafferty *et al.*, *Nature* (1990) 348:552-554; and Griffiths *et al.*, (1993) *EMBO J* 12:725-734. The scFv antibody libraries preferably are screened using recombinant human TNF $\alpha$  as the antigen.

Once initial human VL and VH segments are selected, "mix and match" experiments, in which different pairs of the initially selected VL and VH segments are screened for hTNF $\alpha$  binding, are performed to select preferred VL/VH pair combinations. Additionally, to further improve the affinity and/or lower the off rate constant for hTNF $\alpha$  binding, the VL and VH segments of the preferred VL/VH pair(s) can be randomly mutated, preferably within the CDR3 region of VH and/or VL, in a process analogous to the *in vivo* somatic mutation process responsible for affinity maturation of antibodies during a natural immune response. This *in vitro* affinity maturation can be accomplished by amplifying VH and VL regions

using PCR primers complimentary to the VH CDR3 or VL CDR3, respectively, which primers have been "spiked" with a random mixture of the four nucleotide bases at certain positions such that the resultant PCR products encode VH and VL segments into which random mutations have been introduced into the VH and/or VL CDR3 regions. These 5 randomly mutated VH and VL segments can be rescreened for binding to hTNF $\alpha$  and sequences that exhibit high affinity and a low off rate for hTNF $\alpha$  binding can be selected.

Following screening and isolation of an anti-hTNF $\alpha$  antibody of the invention from a recombinant immunoglobulin display library, nucleic acid encoding the selected antibody can be recovered from the display package (*e.g.*, from the phage genome) and subcloned into 10 other expression vectors by standard recombinant DNA techniques. If desired, the nucleic acid can be further manipulated to create other antibody forms of the invention (*e.g.*, linked to nucleic acid encoding additional immunoglobulin domains, such as additional constant regions). To express a recombinant human antibody isolated by screening of a combinatorial library, the DNA encoding the antibody is cloned into a recombinant expression vector and 15 introduced into a mammalian host cells, as described in further detail in Section II above.

#### IV. Pharmaceutical Compositions and Pharmaceutical Administration

The antibodies and antibody-portions of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject for the methods 20 described herein, *e.g.*, biweekly, subcutaneous dosing. Typically, the pharmaceutical composition comprises an antibody (or antibody portion) of the invention and/or methotrexate and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are 25 physiologically compatible and are suitable for administration to a subject for the methods described herein. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the 30 composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

The compositions of this invention may be in a variety of forms. These include, for 35 example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The preferred mode of administration is parenteral (*e.g.*,

intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular injection. In a particularly preferred embodiment, the antibody is administered by subcutaneous injection (e.g., a 5 biweekly, subcutaneous injection).

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound (i.e., antibody or 10 antibody portion) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the 15 preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can 20 be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

The antibodies and antibody-portions of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is subcutaneous injection. As will be appreciated by the skilled 25 artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyethylene glycol (PEG), 30 polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

In certain embodiments, an antibody or antibody portion of the invention may be 35 orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups,

wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, an antibody or antibody portion of the invention is coformulated with and/or coadministered with one or more additional therapeutic agents. For example, an anti-hTNF $\alpha$  antibody or antibody portion of the invention may be coformulated and/or coadministered with methotrexate, one or more additional antibodies that bind other targets (e.g., antibodies that bind other cytokines or that bind cell surface molecules), one or more cytokines, soluble TNF $\alpha$  receptor (see e.g., PCT Publication No. WO 94/06476) and/or one or more chemical agents that inhibit hTNF $\alpha$  production or activity (such as cyclohexane-ylidene derivatives as described in PCT Publication No. WO 93/19751). Furthermore, one or more antibodies of the invention may be used in combination with two or more of the foregoing therapeutic agents. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies. The use of the antibodies, or antibody portions, of the invention in combination with other therapeutic agents is discussed further in subsection IV.

Non-limiting examples of therapeutic agents for rheumatoid arthritis with which an antibody, or antibody portion, of the invention can be combined include the following: non-steroidal anti-inflammatory drug(s) (NSAIDs); cytokine suppressive anti-inflammatory drug(s) (CSAIDs); CDP-571/BAY-10-3356 (humanized anti-TNF $\alpha$  antibody; Celltech/Bayer); cA2 (chimeric anti-TNF $\alpha$  antibody; Centocor); 75 kdTNFR-IgG (75 kD TNF receptor-IgG fusion protein; Immunex; see e.g., *Arthritis & Rheumatism* (1994) Vol. 37, S295; *J. Invest. Med.* (1996) Vol. 44, 235A); 55 kdTNFR-IgG (55 kD TNF receptor-IgG fusion protein; Hoffmann-LaRoche); IDEC-CE9.1/SB 210396 (non-depleting primatized anti-CD4 antibody; IDEC/SmithKline; see e.g., *Arthritis & Rheumatism* (1995) Vol. 38, S185); DAB 486-IL-2 and/or DAB 389-IL-2 (IL-2 fusion proteins; Seragen; see e.g., *Arthritis & Rheumatism* (1993) Vol. 36, 1223); Anti-Tac (humanized anti-IL-2R $\alpha$ ; Protein Design Labs/Roche); IL-4 (anti-inflammatory cytokine; DNAX/Schering); IL-10 (SCH 52000; recombinant IL-10, anti-inflammatory cytokine; DNAX/Schering); IL-4; IL-10 and/or IL-4 agonists (e.g., agonist antibodies); IL-1RA (IL-1 receptor antagonist; Synergen/Amgen); TNF-bp/s-TNFR (soluble TNF binding protein; see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S284; *Amer. J. Physiol. - Heart and Circulatory Physiology* (1995) Vol. 268, pp. 37-42); R973401 (phosphodiesterase Type IV inhibitor; see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282); MK-966 (COX-2 Inhibitor; see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S81); Illoprost (see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S82); methotrexate; thalidomide (see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282) and thalidomide-

related drugs (e.g., Celgen); leflunomide (anti-inflammatory and cytokine inhibitor; see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S131; *Inflammation Research* (1996) Vol. 45, pp. 103-107); tranexamic acid (inhibitor of plasminogen activation; see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S284); T-614 (cytokine inhibitor; see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282); prostaglandin E1 (see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282); Tenidap (non-steroidal anti-inflammatory drug; see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S280); Naproxen (non-steroidal anti-inflammatory drug; see e.g., *Neuro Report* (1996) Vol. 7, pp. 1209-1213); Meloxicam (non-steroidal anti-inflammatory drug); Ibuprofen (non-steroidal anti-inflammatory drug); Piroxicam (non-steroidal anti-inflammatory drug); Diclofenac (non-steroidal anti-inflammatory drug); Indomethacin (non-steroidal anti-inflammatory drug); Sulfasalazine (see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S281); Azathioprine (see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S281); ICE inhibitor (inhibitor of the enzyme interleukin-1 $\beta$  converting enzyme); zap-70 and/or lck inhibitor (inhibitor of the tyrosine kinase zap-70 or lck); VEGF inhibitor and/or VEGF-R inhibitor (inhibitors of vascular endothelial cell growth factor or vascular endothelial cell growth factor receptor; inhibitors of angiogenesis); corticosteroid anti-inflammatory drugs (e.g., SB203580); TNF-convertase inhibitors; anti-IL-12 antibodies; interleukin-11 (see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S296); interleukin-13 (see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S308); interleukin-17 inhibitors (see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S120); gold; penicillamine; chloroquine; hydroxychloroquine; chlorambucil; cyclophosphamide; cyclosporine; total lymphoid irradiation; anti-thymocyte globulin; anti-CD4 antibodies; CD5-toxins; orally-administered peptides and collagen; lobenzarit disodium; Cytokine Regulating Agents (CRAs) HP228 and HP466 (Houghten Pharmaceuticals, Inc.); ICAM-1 antisense phosphorothioate oligodeoxynucleotides (ISIS 2302; Isis Pharmaceuticals, Inc.); soluble complement receptor 1 (TP10; T Cell Sciences, Inc.); prednisone; orgotein; glycosaminoglycan polysulphate; minocycline; anti-IL2R antibodies; marine and botanical lipids (fish and plant seed fatty acids; see e.g., DeLuca et al. (1995) *Rheum. Dis. Clin. North Am.* 21:759-777); auranofin; phenylbutazone; meclofenamic acid; flufenamic acid; intravenous immune globulin; zileuton; mycophenolic acid (RS-61443); tacrolimus (FK-506); sirolimus (rapamycin); amiprilose (therafectin); cladribine (2-chlorodeoxyadenosine); and azaribine.

Non-limiting examples of therapeutic agents for inflammatory bowel disease with which an antibody, or antibody portion, of the invention can be combined include the following: budesonide; epidermal growth factor; corticosteroids; cyclosporin, sulfasalazine; aminosalicylates; 6-mercaptopurine; azathioprine; metronidazole; lipoxigenase inhibitors; mesalamine; olsalazine; balsalazide; antioxidants; thromboxane inhibitors; IL-1 receptor antagonists; anti-IL-1 $\beta$  monoclonal antibodies; anti-IL-6 monoclonal antibodies; growth

- factors; elastase inhibitors; pyridinyl-imidazole compounds; CDP-571/BAY-10-3356 (humanized anti-TNF $\alpha$  antibody; Celltech/Bayer); cA2 (chimeric anti-TNF $\alpha$  antibody; Centocor); 75 kdTNFR-IgG (75 kD TNF receptor-IgG fusion protein; Immunex; see e.g., *Arthritis & Rheumatism* (1994) Vol. 37, S295; *J. Invest. Med.* (1996) Vol. 44, 235A); 55 kdTNFR-IgG (55 kD TNF receptor-IgG fusion protein; Hoffmann-LaRoche); interleukin-10 (SCH 52000; Schering Plough); IL-4; IL-10 and/or IL-4 agonists (e.g., agonist antibodies); interleukin-11; glucuronide- or dextran-conjugated prodrugs of prednisolone, dexamethasone or budesonide; ICAM-1 antisense phosphorothioate oligodeoxynucleotides (ISIS 2302; Isis Pharmaceuticals, Inc.); soluble complement receptor 1 (TP10; T Cell Sciences, Inc.); slow-release mesalazine; methotrexate; antagonists of Platelet Activating Factor (PAF); ciprofloxacin; and lignocaine.

Nonlimiting examples of therapeutic agents for multiple sclerosis with which an antibody, or antibody portion, of the invention can be combined include the following: corticosteroids; prednisolone; methylprednisolone; azathioprine; cyclophosphamide; cyclosporine; methotrexate; 4-aminopyridine; tizanidine; interferon- $\beta$ 1a (Avonex<sup>TM</sup>; Biogen); interferon- $\beta$ 1b (Betaseron<sup>TM</sup>; Chiron/Berlex); Copolymer 1 (Cop-1; Copaxone<sup>TM</sup>; Teva Pharmaceutical Industries, Inc.); hyperbaric oxygen; intravenous immunoglobulin; clabribine; CDP-571/BAY-10-3356 (humanized anti-TNF $\alpha$  antibody; Celltech/Bayer); cA2 (chimeric anti-TNF $\alpha$  antibody; Centocor); 75 kdTNFR-IgG (75 kD TNF receptor-IgG fusion protein; Immunex; see e.g., *Arthritis & Rheumatism* (1994) Vol. 37, S295; *J. Invest. Med.* (1996) Vol. 44, 235A); 55 kdTNFR-IgG (55 kD TNF receptor-IgG fusion protein; Hoffmann-LaRoche); IL-10; IL-4; and IL-10 and/or IL-4 agonists (e.g., agonist antibodies).

Nonlimiting examples of therapeutic agents for sepsis with which an antibody, or antibody portion, of the invention can be combined include the following: hypertonic saline solutions; antibiotics; intravenous gamma globulin; continuous hemofiltration; carbapenems (e.g., meropenem); antagonists of cytokines such as TNF $\alpha$ , IL-1 $\beta$ , IL-6 and/or IL-8; CDP-571/BAY-10-3356 (humanized anti-TNF $\alpha$  antibody; Celltech/Bayer); cA2 (chimeric anti-TNF $\alpha$  antibody; Centocor); 75 kdTNFR-IgG (75 kD TNF receptor-IgG fusion protein; Immunex; see e.g., *Arthritis & Rheumatism* (1994) Vol. 37, S295; *J. Invest. Med.* (1996) Vol. 44, 235A); 55 kdTNFR-IgG (55 kD TNF receptor-IgG fusion protein; Hoffmann-LaRoche); Cytokine Regulating Agents (CRAs) HP228 and HP466 (Houghten Pharmaceuticals, Inc.); SK&F 107647 (low molecular peptide; SmithKline Beecham); tetravalent guanylhydrazone CNI-1493 (Picower Institute); Tissue Factor Pathway Inhibitor (TFPI; Chiron); PHP (chemically modified hemoglobin; APEX Bioscience); iron chelators and chelates, including diethylenetriamine pentaacetic acid - iron (III) complex (DTPA iron (III); Molichem Medicines); lisofylline (synthetic small molecule methylxanthine; Cell Therapeutics, Inc.); PGG-Glucan (aqueous soluble  $\beta$ 1,3glucan; Alpha-Beta Technology); apolipoprotein A-1 reconstituted with lipids; chiral hydroxamic acids (synthetic antibacterials that inhibit lipid A biosynthesis); anti-endotoxin antibodies; E5531 (synthetic lipid A antagonist; Eisai America,

Inc.); rBPI<sub>21</sub> (recombinant N-terminal fragment of human Bactericidal/Permeability-Increasing Protein); and Synthetic Anti-Endotoxin Peptides (SAEP; Bios Ynth Research Laboratories);

Nonlimiting examples of therapeutic agents for adult respiratory distress syndrome (ARDS) with which an antibody, or antibody portion, of the invention can be combined include the following: anti-IL-8 antibodies; surfactant replacement therapy; CDP-571/BAY-10-3356 (humanized anti-TNF $\alpha$  antibody; Celltech/Bayer); cA2 (chimeric anti-TNF $\alpha$  antibody; Centocor); 75 kdTNFR-IgG (75 kD TNF receptor-IgG fusion protein; Immunex; see e.g., *Arthritis & Rheumatism* (1994) Vol. 37, S295; *J. Invest. Med.* (1996) Vol. 44, 235A); and 55 kdTNFR-IgG (55 kD TNF receptor-IgG fusion protein; Hoffmann-LaRoche).

The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antibody portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody or antibody portion may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody or antibody portion of the invention is 10-100 mg, more preferably 20-80 mg and most preferably about 40 mg. It is to be noted that dosage values may vary with

the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary  
5 only and are not intended to limit the scope or practice of the claimed composition.

#### V. Uses of the Antibodies of the Invention

Given their ability to bind to hTNF $\alpha$ , the anti-hTNF $\alpha$  antibodies, or portions thereof, of the invention can be used to detect hTNF $\alpha$  (e.g., in a biological sample, such as serum or plasma), using a conventional immunoassay, such as an enzyme linked immunosorbent assays (ELISA), an radioimmunoassay (RIA) or tissue immunohistochemistry. The invention provides a method for detecting hTNF $\alpha$  in a biological sample comprising contacting a biological sample with an antibody, or antibody portion, of the invention and detecting either the antibody (or antibody portion) bound to hTNF $\alpha$  or unbound antibody (or antibody portion), to thereby detect hTNF $\alpha$  in the biological sample. The antibody is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

Alternative to labeling the antibody, hTNF $\alpha$  can be assayed in biological fluids by a competition immunoassay utilizing rhTNF $\alpha$  standards labeled with a detectable substance and an unlabeled anti-hTNF $\alpha$  antibody. In this assay, the biological sample, the labeled rhTNF $\alpha$  standards and the anti-hTNF $\alpha$  antibody are combined and the amount of labeled rhTNF $\alpha$  standard bound to the unlabeled antibody is determined. The amount of hTNF $\alpha$  in the biological sample is inversely proportional to the amount of labeled hTNF $\alpha$  standard bound to the anti-hTNF $\alpha$  antibody.

A D2E7 antibody of the invention can also be used to detect TNF $\alpha$ s from species other than humans, in particular TNF $\alpha$ s from primates (e.g., chimpanzee, baboon, marmoset, cynomolgus and rhesus), pig and mouse, since D2E7 can bind to each of these TNF $\alpha$ s.

The antibodies and antibody portions of the invention are capable of neutralizing hTNF $\alpha$  activity both *in vitro* and *in vivo* (see U.S. Patent No. 6,090,382). Moreover, at least some of the antibodies of the invention, such as D2E7, can neutralize hTNF $\alpha$  activity from other species. Accordingly, the antibodies and antibody portions of the invention can be used to inhibit hTNF $\alpha$  activity, e.g., in a cell culture containing hTNF $\alpha$ , in human subjects or in

other mammalian subjects having TNF $\alpha$ s with which an antibody of the invention cross-reacts (e.g. chimpanzee, baboon, marmoset, cynomolgus and rhesus, pig or mouse). In one embodiment, the invention provides a method for inhibiting TNF $\alpha$  activity comprising contacting TNF $\alpha$  with an antibody or antibody portion of the invention such that TNF $\alpha$  activity is inhibited. Preferably, the TNF $\alpha$  is human TNF $\alpha$ . For example, in a cell culture containing, or suspected of containing TNF $\alpha$ , an antibody or antibody portion of the invention can be added to the culture medium to inhibit hTNF $\alpha$  activity in the culture.

In a preferred embodiment, the invention provides methods of treating disorders in which the administration of an anti-TNF $\alpha$  antibody is beneficial, comprising subcutaneously administering to the subject biweekly an antibody or antibody portion of the invention such that the disorder is treated. In a particularly preferred embodiment, the antibody is administered subcutaneously on a biweekly schedule. In another particularly preferred embodiment, the antibody is administered subcutaneously before, during or after administration of methotrexate. Preferably, the subject is a human subject. Alternatively, the subject can be a mammal expressing a TNF $\alpha$  with which an antibody of the invention cross-reacts. Still further the subject can be a mammal into which has been introduced hTNF $\alpha$  (e.g., by administration of hTNF $\alpha$  or by expression of an hTNF $\alpha$  transgene). An antibody of the invention can be administered to a human subject for therapeutic purposes (discussed further below). Moreover, an antibody of the invention can be administered to a non-human mammal expressing a TNF $\alpha$  with which the antibody cross-reacts (e.g., a primate, pig or mouse) for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of antibodies of the invention (e.g., testing of dosages and time courses of administration).

As used herein, the term "a disorder in which the administration of an anti-TNF $\alpha$  antibody is beneficial" is intended to include diseases and other disorders in which the presence of TNF $\alpha$  in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder, or where it has been shown that another anti-TNF $\alpha$  antibody or a biologically active portion thereof has been successfully used to treat the disease. Accordingly, a disorder in which TNF $\alpha$  activity is detrimental is a disorder in which inhibition of TNF $\alpha$  activity is expected to alleviate the symptoms and/or progression of the disorder. Such disorders may be evidenced, for example, by an increase in the concentration of TNF $\alpha$  in a biological fluid of a subject suffering from the disorder (e.g., an increase in the concentration of TNF $\alpha$  in serum, plasma, synovial fluid, etc. of the subject), which can be detected, for example, using an anti-TNF $\alpha$  antibody as described above. There are numerous examples of disorders in which TNF $\alpha$  activity is detrimental. The use of the antibodies and antibody portions of the invention in the treatment of specific disorders is discussed further below:

**A. Sepsis**

- Tumor necrosis factor has an established role in the pathophysiology of sepsis, with biological effects that include hypotension, myocardial suppression, vascular leakage syndrome, organ necrosis, stimulation of the release of toxic secondary mediators and activation of the clotting cascade (see e.g., Tracey, K.J. and Cerami, A. (1994) *Annu. Rev. Med.* **45**:491-503; Russell, D and Thompson, R.C. (1993) *Curr. Opin. Biotech.* **4**:714-721). Accordingly, the human antibodies, and antibody portions, of the invention can be used to treat sepsis in any of its clinical settings, including septic shock, endotoxic shock, gram negative sepsis and toxic shock syndrome.
- Furthermore, to treat sepsis, an anti-hTNF $\alpha$  antibody, or antibody portion, of the invention can be coadministered with one or more additional therapeutic agents that may further alleviate sepsis, such as an interleukin-1 inhibitor (such as those described in PCT Publication Nos. WO 92/16221 and WO 92/17583), the cytokine interleukin-6 (see e.g., PCT Publication No. WO 93/11793) or an antagonist of platelet activating factor (see e.g., European Patent Application Publication No. EP 374 510).

Additionally, in a preferred embodiment, an anti-TNF $\alpha$  antibody or antibody portion of the invention is administered to a human subject within a subgroup of sepsis patients having a serum or plasma concentration of IL-6 above 500 pg/ml, and more preferably 1000 pg/ml, at the time of treatment (see PCT Publication No. WO 95/20978 by Daum, L., et al.).

**20 B. Autoimmune Diseases**

- Tumor necrosis factor has been implicated in playing a role in the pathophysiology of a variety of autoimmune diseases. For example, TNF $\alpha$  has been implicated in activating tissue inflammation and causing joint destruction in rheumatoid arthritis (see e.g., Tracey and Cerami, *supra*; Arend, W.P. and Dayer, J-M. (1995) *Arth. Rheum.* **38**:151-160; Fava, R.A., et al. (1993) *Clin. Exp. Immunol.* **94**:261-266). TNF $\alpha$  also has been implicated in promoting the death of islet cells and in mediating insulin resistance in diabetes (see e.g., Tracey and Cerami, *supra*; PCT Publication No. WO 94/08609). TNF $\alpha$  also has been implicated in mediating cytotoxicity to oligodendrocytes and induction of inflammatory plaques in multiple sclerosis (see e.g., Tracey and Cerami, *supra*). Chimeric and humanized murine anti-hTNF $\alpha$  antibodies have undergone clinical testing for treatment of rheumatoid arthritis (see e.g., Elliott, M.J., et al. (1994) *Lancet* **344**:1125-1127; Elliot, M.J., et al. (1994) *Lancet* **344**:1105-1110; Rankin, E.C., et al. (1995) *Br. J. Rheumatol.* **34**:334-342).

The human antibodies, and antibody portions of the invention can be used to treat autoimmune diseases, in particular those associated with inflammation, including rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis and gouty arthritis, allergy, multiple sclerosis, autoimmune diabetes, autoimmune uveitis and nephrotic syndrome. Typically, the antibody, or antibody portion, is administered systemically, although for certain disorders, local administration of the antibody or antibody portion at a site of inflammation may be beneficial (e.g., local administration in the joints in rheumatoid arthritis or topical application to

diabetic ulcers, alone or in combination with a cyclohexane-ylidene derivative as described in PCT Publication No. WO 93/19751).

### C. Infectious Diseases

Tumor necrosis factor has been implicated in mediating biological effects observed in 5 a variety of infectious diseases. For example, TNF $\alpha$  has been implicated in mediating brain inflammation and capillary thrombosis and infarction in malaria (see e.g., Tracey and Cerami, *supra*). TNF $\alpha$  also has been implicated in mediating brain inflammation, inducing breakdown of the blood-brain barrier, triggering septic shock syndrome and activating venous infarction in meningitis (see e.g., Tracey and Cerami, *supra*). TNF $\alpha$  also has been implicated 10 in inducing cachexia, stimulating viral proliferation and mediating central nervous system injury in acquired immune deficiency syndrome (AIDS) (see e.g., Tracey and Cerami, *supra*). Accordingly, the antibodies, and antibody portions, of the invention, can be used in the treatment of infectious diseases, including bacterial meningitis (see e.g., European Patent Application Publication No. EP 585 705), cerebral malaria, AIDS and AIDS-related complex 15 (ARC) (see e.g., European Patent Application Publication No. EP 230 574), as well as cytomegalovirus infection secondary to transplantation (see e.g., Fietze, E., et al. (1994) *Transplantation* 58:675-680). The antibodies, and antibody portions, of the invention, also can be used to alleviate symptoms associated with infectious diseases, including fever and 20 myalgias due to infection (such as influenza) and cachexia secondary to infection (e.g., secondary to AIDS or ARC).

### D. Transplantation

Tumor necrosis factor has been implicated as a key mediator of allograft rejection and graft versus host disease (GVHD) and in mediating an adverse reaction that has been observed when the rat antibody OKT3, directed against the T cell receptor CD3 complex, is 25 used to inhibit rejection of renal transplants (see e.g., Tracey and Cerami, *supra*; Eason, J.D., et al. (1995) *Transplantation* 59:300-305; Suthanthiran, M. and Strom, T.B. (1994) *New Engl. J. Med.* 331:365-375). Accordingly, the antibodies, and antibody portions, of the invention, can be used to inhibit transplant rejection, including rejections of allografts and xenografts and to inhibit GVHD. Although the antibody or antibody portion may be used 30 alone, more preferably it is used in combination with one or more other agents that inhibit the immune response against the allograft or inhibit GVHD. For example, in one embodiment, an antibody or antibody portion of the invention is used in combination with OKT3 to inhibit OKT3-induced reactions. In another embodiment, an antibody or antibody portion of the invention is used in combination with one or more antibodies directed at other targets 35 involved in regulating immune responses, such as the cell surface molecules CD25 (interleukin-2 receptor- $\alpha$ ), CD11a (LFA-1), CD54 (ICAM-1), CD4, CD45, CD28/CTLA4, CD80 (B7-1) and/or CD86 (B7-2). In yet another embodiment, an antibody or antibody portion of the invention is used in combination with one or more general immunosuppressive agents, such as cyclosporin A or FK506.

#### E. Malignancy

Tumor necrosis factor has been implicated in inducing cachexia, stimulating tumor growth, enhancing metastatic potential and mediating cytotoxicity in malignancies (see e.g., Tracey and Cerami, *supra*). Accordingly, the antibodies, and antibody portions, of the invention, can be used in the treatment of malignancies, to inhibit tumor growth or metastasis and/or to alleviate cachexia secondary to malignancy. The antibody, or antibody portion, may be administered systemically or locally to the tumor site.

#### F. Pulmonary Disorders

Tumor necrosis factor has been implicated in the pathophysiology of adult respiratory distress syndrome, including stimulating leukocyte-endothelial activation, directing cytotoxicity to pneumocytes and inducing vascular leakage syndrome (see e.g., Tracey and Cerami, *supra*). Accordingly, the antibodies, and antibody portions, of the invention, can be used to treat various pulmonary disorders, including adult respiratory distress syndrome (see e.g., PCT Publication No. WO 91/04054), shock lung, chronic pulmonary inflammatory disease, pulmonary sarcoidosis, pulmonary fibrosis and silicosis. The antibody, or antibody portion, may be administered systemically or locally to the lung surface, for example as an aerosol.

#### G. Intestinal Disorders

Tumor necrosis factor has been implicated in the pathophysiology of inflammatory bowel disorders (see e.g., Tracy, K.J., *et al.* (1986) *Science* 234:470-474; Sun, X-M., *et al.* (1988) *J. Clin. Invest.* 81:1328-1331; MacDonald, T.T., *et al.* (1990) *Clin. Exp. Immunol.* 81:301-305). Chimeric murine anti-hTNF $\alpha$  antibodies have undergone clinical testing for treatment of Crohn's disease (van Dullemen, H.M., *et al.* (1995) *Gastroenterology* 109:129-135). The human antibodies, and antibody portions, of the invention, also can be used to treat intestinal disorders, such as idiopathic inflammatory bowel disease, which includes two syndromes, Crohn's disease and ulcerative colitis.

#### H. Cardiac Disorders

The antibodies, and antibody portions, of the invention, also can be used to treat various cardiac disorders, including ischemia of the heart (see e.g., European Patent Application Publication No. EP 453 898) and heart insufficiency (weakness of the heart muscle) (see e.g., PCT Publication No. WO 94/20139).

#### I. Others

The antibodies, and antibody portions, of the invention, also can be used to treat various other disorders in which TNF $\alpha$  activity is detrimental. Examples of other diseases and disorders in which TNF $\alpha$  activity has been implicated in the pathophysiology, and thus which can be treated using an antibody, or antibody portion, of the invention, include inflammatory bone disorders and bone resorption disease (see e.g., Bertolini, D.R., *et al.* (1986) *Nature* 319:516-518; Konig, A., *et al.* (1988) *J. Bone Miner. Res.* 3:621-627; Lerner, U.H. and Ohlin, A. (1993) *J. Bone Miner. Res.* 8:147-155; and Shankar, G. and Stern, P.H.

(1993) *Bone* 14:871-876), hepatitis, including alcoholic hepatitis (see e.g., McClain, C.J. and Cohen, D.A. (1989) *Hepatology* 9:349-351; Felver, M.E., et al. (1990) *Alcohol. Clin. Exp. Res.* 14:255-259; and Hansen, J., et al. (1994) *Hepatology* 20:461-474) and viral hepatitis (Sheron, N., et al. (1991) *J. Hepatol.* 12:241-245; and Hussain, M.J., et al. (1994) *J. Clin. Pathol.* 47:1112-1115), coagulation disturbances (see e.g., van der Poll, T., et al. (1990) *N. Engl. J. Med.* 322:1622-1627; and van der Poll, T., et al. (1991) *Prog. Clin. Biol. Res.* 367:55-60), burns (see e.g., Giroir, B.P., et al. (1994) *Am. J. Physiol.* 267:H118-124; and Liu, X.S., et al. (1994) *Burns* 20:40-44), reperfusion injury (see e.g., Scales, W.E., et al. (1994) *Am. J. Physiol.* 267:G1122-1127; Serrick, C., et al. (1994) *Transplantation* 58:1158-1162; and Yao, Y.M., et al. (1995) *Resuscitation* 29:157-168), keloid formation (see e.g., McCauley, R.L., et al. (1992) *J. Clin. Immunol.* 12:300-308), scar tissue formation and pyrexia.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are hereby incorporated by reference.

**EXAMPLE 1:** Treatment With An Anti-TNF $\alpha$  Antibody

**D2E7 Efficacy Following Subcutaneous Administration**

In this study, twenty-four patients with active RA were treated with weekly doses of 0.5 mg/kg D2E7 (n=18) or placebo (n=6) by s.c. injection for three months. Patients participating in this study had a mean duration of disease of 10.1 years with a disease activity score (DAS) score of 4.87 and a mean of 3.4 DMARDs (disease modifying anti-rheumatic drugs) prior to study entry; again reflecting considerable disease activity. Responders continued open-label treatment with D2E7, while patients who failed to respond to the 0.5 mg/kg dose or who lost a DAS response on the 0.5 mg/kg dose were escalated to receive 1 mg/kg by s.c. injection after week twelve of the study.

The first patients enrolled received up to sixty injections and were, therefore, sixty weeks on the study drug. The efficacy with s.c. dosing was similar to i.v. injections. Up to 78% of patients reached a DAS and ACR20 response during the first weeks of treatment. Subcutaneous D2E7 at a dose of 0.5 mg/kg/week reduced the swollen joint (SWJ) count by 54%, tender joint count (TJC) by 61% and CRP by 39% over twelve weeks compared to baseline, whereas all parameters increased in the placebo group. After completion of the placebo-controlled period of this study, the patients continued treatment for up to fourteen months with sustained efficacy. These results indicate that subcutaneous D2E7 at a dose of 0.5 mg/kg/week can, therefore, be safely self-administered with good local tolerability.

**Administration Of D2E7 And Methotrexate**

In this study, patients received s.c. or i.v. placebo or D2E7 at a dose of 1 mg/kg in addition to their ongoing treatment with (methotrexate) MTX. Fifty-four patients were enrolled in the study and eighteen patients received i.v. D2E7 and s.c. placebo, eighteen

patients received i.v. placebo and s.c. D2E7, and eighteen patients received placebo i.v. and s.c. The patients received their second dose only after they lost their blinded response status, not earlier than four weeks after the first dose. Thereafter, all patients received open-label biweekly s.c. injections of D2E7.

5 Demographic characteristics of the study population of this study included a mean duration of RA of 11.1 years, prior exposure to a mean of 3.6 DMARDs (other than MTX), and a mean DAS at study entry of 4.81. By Day twenty-nine, 72% of the i.v. D2E7 treated patients and 44% of the s.c. D2E7 treated patients had achieved a response by DAS criteria, compared to only 28% of placebo-treated patients (set forth in Figure 5). Of the responders  
10 in this study, 28% of placebo treated patients maintained an ACR20 response up to day 29, compared to 72% of i.v.-treated D2E7 patients and 67% of s.c.-treated D2E7 patients, who maintained their responses for between one and three months.

**EXAMPLE 2: Total Body Dose Of A Subcutaneously Administered Anti-TNF $\alpha$**

15 **Antibody**

**Weekly, Subcutaneous Administration of D2E7**

This study enrolled two hundred eighty-four patients with RA and was designed to determine the optimal total body dose of subcutaneously administered D2E7. Patients were randomized to receive either 20, 40, or 80 mg D2E7 or placebo weekly for twelve weeks,  
20 after which time placebo-treated patients were switched blindly to 40 mg D2E7/week.

Approximately 49% of patients reached ACR20 at 20 mg, 55% of patients reached ACR20 at 40 mg, and 54% of patients reached ACR20 at 80 mg, while only 10% of patients receiving placebo reached ACR20 (set forth in Figure 1A). Approximately 23% of patients reached ACR50 at 20 mg, 27% of patients reached ACR50 at 40 mg, and 20% of patients  
25 reached ACR50 at 80 mg, and only 2% of patients receiving placebo reached ACR50. These data illustrate that subcutaneous D2E7, particularly at a dose of 40 mg/week, generates a good response.

**EXAMPLE 3: Biweekly, Subcutaneous Administration Of An Anti-TNF $\alpha$  Antibody**

**Biweekly, Subcutaneous Administration Of D2E7**

30 The clinical effects, safety, immunogenicity, and tolerance of RA patients with partial responses to MTX following every other week subcutaneous (s.c.) injections of placebo or D2E7 at several dose levels for up to twenty-four weeks in conjunction with continued MTX treatment was investigated.

**Study Design**

35 A placebo-controlled, double-blind, randomized, multi-center study in patients with RA, who had insufficient efficacy or tolerability to MTX was performed. During the course of the trial, patients were continued on a stable dose of MTX with dose ranges specified in the inclusion criteria described below.

- This study consisted of two portions: 1) a "wash-out period" of four weeks prior to the administration of the first dose medication, during which time DMARDs (except for MTX) were withdrawn; and 2) a "placebo controlled period" during which time patients were randomized to one of four cohorts of sixty-seven patients to receive placebo, 20, 40, or 80 mg D2E7 (as a total body dose) given every other week s.c. for up to 24 weeks. Each dose of study drug was administered as two s.c. injections of 1.6 mL each. The patient's first dose was administered by medical personnel as part of the patient's training. Subsequent doses were self-administered by the patient at the study under the direct observation of trained personnel for the first four weeks. Thereafter, doses were administered outside the study site by the patient, a trained individual designated by the patient, or by medical personnel.
- Medication for four or five weeks was dispensed after each clinical assessment. Patients were serially examined in weeks one, two, three, four, six, eight, twelve, sixteen, twenty, and twenty-four of the study with the joint examinations being performed by a blinded assessor, independent of the treating physician.
- This study enrolled two hundred seventy-one patients with RA. The study population was representative of the moderate to severe RA population in North America: approximately 70% female, and predominantly over the age of forty. The population was selected using predetermined inclusion and exclusion criteria, known to those of skill in the art e.g., a patient must have received a diagnosis of RA as defined by the 1987-revised American College of Rheumatology (ACR) criteria (set forth in Appendix A).

#### Results

Figures 1B and 2-4 indicate that subcutaneous, biweekly D2E7 treatment combined with methotrexate was significantly better than placebo in reducing the signs and symptoms of RA at twenty-four weeks. All three doses of D2E7 were statistically significantly more effective than placebo given weekly. Furthermore, D2E7 at 40 mg and 80 mg had better efficacy than the 20 mg dose.

#### EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

### ACR Definition of RA

The 1987 classification tree criteria and functions for rheumatoid arthritis (RA)

CRITERION	DEFINITION
1. Arthritis of 3 or more joint areas	At least 3 joint areas simultaneously have had soft tissues swelling or fluid (not bony overgrowth alone) observed by a physician. The 14 possible joint areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints.
2. Arthritis of hand joints Wrist MCP MCP or wrist MCP and wrist	Soft tissue swelling or fluid (not bony overgrowth alone) of the specified area observed by a physician. Where 2 areas are specified, involvement must have been simultaneous.
3. Symmetric swelling (arthritis)	Simultaneous involvement of the same joint areas (as defined in 1 on both sides of the body (bilateral involvement of PIPs, MCP's, or MTPs is acceptable without absolute symmetry)
4. Serum rheumatoid factor.	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects
5. Radiographic changes of rheumatoid arthritis	Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (Osteoarthritis changes alone do not qualify)

- A patient is said to have RA if he/she is included in 1 of the SRA subsets listed in Table 7 and has a clinical diagnosis of RA by his/her physician. Criteria 1, 2, and 3 must have been present for at least 6 weeks.

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### APPENDIX A

## SEQ ID NO:1:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Tyr  
 20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
 35 40 45

Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
 65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Gln Arg Tyr Asn Arg Ala Pro Tyr  
 85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
 100 105

## SEQ ID NO:2:

Glu Val Gln Leu Val Glu Ser Gly Gly Leu Val Gln Pro Gly Arg  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr  
 20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45

Ser Ala Ile Thr Trp Asn Ser Gly His Ile Asp Tyr Ala Asp Ser Val  
 50 55 60

Glu Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr  
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Lys Val Ser Tyr Leu Ser Thr Ala Ser Ser Leu Asp Tyr Trp Gly  
 100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser  
 115 120

## SEQ ID NO:3:

Gln Arg Tyr Asn Arg Ala Pro Tyr Xaa  
 1 5

**APPENDIX B**

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr  
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Asp Trp Val  
35 40 45

Ser Ala Ile Thr Trp Asn Ser Gly His Ile Asp Tyr Ala Asp Ser Val  
50 55 60

Glu Gly Arg Phe Ala Val Ser Arg Asp Asn Ala Lys Asn Ala Leu Tyr  
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Thr Lys Ala Ser Tyr Leu Ser Thr Ser Ser Ser Leu Asp Asn Trp Gly  
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser  
115 120

## SEQ ID NO:11:

Gln Lys Tyr Asn Ser Ala Pro Tyr Ala  
1 5

## SEQ ID NO:12:

Gln Lys Tyr Asn Arg Ala Pro Tyr Ala  
1 5

## SEQ ID NO:13:

Gln Lys Tyr Gln Arg Ala Pro Tyr Thr  
1 5

## SEQ ID NO:14:

Gln Lys Tyr Ser Ser Ala Pro Tyr Thr  
1 5

## SEQ ID NO:15:

Gln Lys Tyr Asn Ser Ala Pro Tyr Thr  
1 5

## SEQ ID NO:16:

Gln Lys Tyr Asn Arg Ala Pro Tyr Thr

SEQ ID NO:17:

Gln Lys Tyr Asn Ser Ala Pro Tyr Tyr  
1 5

SEQ ID NO:18:

Gln Lys Tyr Asn Ser Ala Pro Tyr Asn  
1 5

SEQ ID NO:19:

Gln Lys Tyr Thr Ser Ala Pro Tyr Thr  
1 5

SEQ ID NO:20:

Gln Lys Tyr Asn Arg Ala Pro Tyr Asn  
1 5

SEQ ID NO:21:

Gln Lys Tyr Asn Ser Ala Ala Tyr Ser  
1 5

SEQ ID NO:22:

Gln Gln Tyr Asn Ser Ala Pro Asp Thr  
1 5

SEQ ID NO:23:

Gln Lys Tyr Asn Ser Asp Pro Tyr Thr  
1 5

SEQ ID NO:24:

Gln Lys Tyr Ile Ser Ala Pro Tyr Thr  
1 5

SEQ ID NO:25:

Gln Lys Tyr Asn Arg Pro Pro Tyr Thr  
1 5

SEQ ID NO:26:

SEQ ID NO:27:

Ala Ser Tyr Leu Ser Thr Ser Ser Ser Leu Asp Asn  
1 5 10

SEQ ID NO:28:

Ala Ser Tyr Leu Ser Thr Ser Ser Ser Leu Asp Lys  
1 5 10

SEQ ID NO:29:

Ala Ser Tyr Leu Ser Thr Ser Ser Ser Leu Asp Tyr  
1 5 10

SEQ ID NO:30:

Ala Ser Tyr Leu Ser Thr Ser Ser Ser Leu Asp Asp  
1 5 10

SEQ ID NO:31:

Ala Ser Tyr Leu Ser Thr Ser Phe Ser Leu Asp Tyr  
1 5 10

SEQ ID NO:32:

Ala Ser Tyr Leu Ser Thr Ser Ser Ser Leu His Tyr  
1 5 10

SEQ ID NO:33:

Ala Ser Phe Leu Ser Thr Ser Ser Ser Leu Glu Tyr  
1 5 10

SEQ ID NO:34:

Ala Ser Tyr Leu Ser Thr Ala Ser Ser Leu Glu Tyr  
1 5 10

SEQ ID NO:35:

Val Ser Tyr Leu Ser Thr Ala Ser Ser Leu Asp Asn  
1 5 10

GACATCCAGA TGACCCAGTC TCCATCCTCC CTGTCTGCAT CTGTAGGGGA CAGAGTCACC	60
ATCACTTGTC GGGCAAGTCA GGGCATCAGA AATTACCTTAG CCTGGTATCA GCAAAAACCA	120
GGGAAAGCCC CTAAGCTCCT GATCTATGCT GCATCCACTT TGCAATCAGG GGTCCCATCT	180
CGGTTCACTG GCAGTGGATC TGGGACAGAT TTCACTCTCA CCATCAGCAG CCTACAGCCT	240
GAAGATGTTG CAACTTATTA CTGTCAAAGG TATAACCGTG CACCGTATAC TTTTGGCCAG	300
GGGACCAAGG TGGAAATCAA A	321

SEQ ID NO:37:

GAGGTGCAGC TGGTGGAGTC TGGGGGAGGC TTGGTACAGC CCGGCAGGTC CCTGAGACTC	60
TCCTGTGCGG CCTCTGGATT CACCTTGAT GATTATGCCA TGCAGTGGGT CCCGCAAGCT	120
CCAGGGAAGG GCCTGGAATG GGTCTCAGCT ATCACTTGGA ATAGTGGTCA CATAGACTAT	180
CGGGACTCTG TGGAGGGCCG ATTCAACCATC TCCAGAGACA ACGCCAAGAA CTCCCTGTAT	240
CTGCAAATGA ACAGTCTGAG AGCTGAGGAT ACGGCCGTAT ATTACTGTGC GAAAGTCTCG	300
TACCTTAGCA CGCGTCCTC CCTTGACTAT TGGGGCCAAG GTACCCCTGGT CACCGTCTCG	360
AGT	363

CLAIMS

What is claimed:

1. A method for treating a disorder in a human subject in which the disease is treatable with a TNF $\alpha$  antibody, comprising administering a composition to the human subject in need thereof, on a biweekly dosing regimen such that the disorder is treated, said composition containing an anti-TNF $\alpha$  antibody or an antigen binding portion thereof.
2. The method of claim 1, wherein the administration is by subcutaneous injection.
3. The method of claim 1, wherein said anti-TNF $\alpha$  antibody or an antigen binding portion thereof is a human anti-TNF $\alpha$  antibody.
4. The method of claim 3, wherein said human antibody, or an antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_d$  of  $1 \times 10^{-8}$  M or less and a  $K_{off}$  rate constant of  $1 \times 10^{-3} \text{ s}^{-1}$  or less, both determined by surface plasmon resonance, and neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC<sub>50</sub> of  $1 \times 10^{-7}$  M or less.
5. The method of claim 3, wherein said human antibody, or antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  rate constant of  $5 \times 10^{-4} \text{ s}^{-1}$  or less.
6. The method of claim 3, wherein said human antibody, or antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  rate constant of  $1 \times 10^{-4} \text{ s}^{-1}$  or less.
7. The method of claim 3, wherein said human antibody, or antigen-binding portion thereof, neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC<sub>50</sub> of  $1 \times 10^{-8}$  M or less.
8. The method of claim 3, wherein said human antibody, or antigen-binding portion thereof, neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC<sub>50</sub> of  $1 \times 10^{-9}$  M or less.
9. The method of claim 3, wherein said human antibody, or antigen-binding portion thereof, neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC<sub>50</sub> of  $1 \times 10^{-10}$  M or less.

10. The method of claim 3, wherein said human antibody, or antigen-binding portion thereof, is a recombinant antibody, or recombinant antigen-binding portion thereof.

5 11. A method for inhibiting human TNF $\alpha$  activity in a human subject suffering from a disorder in which TNF $\alpha$  activity is detrimental, comprising administering a composition to the human subject on a biweekly dosing regimen, said composition containing a human anti-TNF $\alpha$  antibody wherein said human antibody, or antigen-binding portion thereof, has the following characteristics:

10 a) dissociates from human TNF $\alpha$  with a  $K_{off}$  rate constant of  $1 \times 10^{-3} \text{ s}^{-1}$  or less, as determined by surface plasmon resonance;

b) has a light chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8 or by one to five conservative amino acid substitutions at positions 1, 3, 4, 6, 7, 8 and/or 15 9;

c) has a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 or by one to five conservative amino acid substitutions at positions 2, 3, 4, 5, 6, 8, 9, 10, 11 and/or 12.

20 12. The method of claim 11, wherein the administering is subcutaneously.

25 13. The method of claim 11, wherein said human antibody, or an antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  rate constant of  $5 \times 10^{-4} \text{ s}^{-1}$  or less.

30 14. A method for inhibiting human TNF $\alpha$  activity in a human subject suffering from a disorder in which TNF $\alpha$  activity is detrimental, comprising administering a composition to the human subject, subcutaneously on a biweekly dosing regimen, said composition containing a human anti-TNF $\alpha$  antibody wherein said human antibody, or an antigen-binding portion thereof, has a light chain variable region (LCVR) having a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8, and has a heavy chain variable region (HCVR) having a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11.

35 15. The method of claim 14, wherein the LCVR of said human antibody, or an antigen-binding portion thereof, further has a CDR2 domain comprising the amino acid

sequence of SEQ ID NO: 5 and the HCVR further has a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 6.

16. The method of claim 14, wherein the LCVR of said human antibody, or an  
5 antigen-binding portion thereof, further has CDR1 domain comprising the amino acid  
sequence of SEQ ID NO: 7 and the HCVR has a CDR1 domain comprising the amino acid  
sequence of SEQ ID NO: 8.

17. A method for inhibiting human TNF $\alpha$  activity in a human subject suffering  
10 from a disorder in which TNF $\alpha$  activity is detrimental, comprising administering a  
composition to the human subject, subcutaneously on a biweekly dosing regimen, said  
composition containing a human anti-TNF $\alpha$  antibody wherein said human antibody, or an  
antigen binding portion thereof, has a light chain variable region (LCVR) comprising the  
amino acid sequence of SEQ ID NO: 1 and a heavy chain variable region (HCVR)  
15 comprising the amino acid sequence of SEQ ID NO: 2.

18. The method of claim 17, wherein said human antibody has an IgG1 heavy  
chain constant region.

20 19. The method of claim 17, wherein said human antibody has an IgG4 heavy  
chain constant region.

20. The method of claim 17, wherein said human antibody is a Fab fragment.

25 21. The method of claim 17, wherein said human antibody is a single chain Fv  
fragment.

22. A method for inhibiting human TNF $\alpha$  activity in a human subject suffering  
from a disorder in which TNF $\alpha$  activity is detrimental, comprising administering a  
30 composition to the human subject, subcutaneously on a biweekly dosing regimen, said  
composition containing a human anti-TNF $\alpha$  antibody wherein said human antibody, or an  
antigen-binding portion thereof, has a light chain variable region (LCVR) having a CDR3  
domain comprising an amino acid sequence selected from the group consisting of SEQ ID  
NO: 3, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15,  
35 SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20; SEQ  
ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO:  
26 or has a heavy chain variable region (HCVR) having a CDR3 domain comprising an  
amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 27,

SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34.

23. A method for inhibiting human TNF $\alpha$  activity in a human subject suffering  
5 from a disorder in which TNF $\alpha$  activity is detrimental, comprising administering a composition to the human subject, subcutaneously on a biweekly dosing regimen, said composition containing a human anti-TNF $\alpha$  antibody wherein said human antibody is the antibody D2E7 or an antigen-binding portion thereof.

10 24. A kit containing a formulation comprising:  
a) a pharmaceutical composition comprising an anti-TNF $\alpha$  antibody and a pharmaceutically acceptable carrier; and  
b) instructions for biweekly dosing of the pharmaceutical composition for the treatment of a disorder in which an anti-TNF $\alpha$  antibody or a binding portion thereof is  
15 effective in treating the disorder.

25. The kit of claim 24, wherein said antibody comprises the antibody, or antigen-binding portion thereof, as set forth in any one of claims 2-22.

20 26. A preloaded syringe containing a pharmaceutical composition comprising an anti-TNF $\alpha$  antibody and a pharmaceutically acceptable carrier.

25 27. The syringe of claim 26, wherein said human antibody comprises the antibody, or antigen-binding portion thereof, as set forth in any one of claims 2-22.

30 28. A method for treating a disorder in which an anti-TNF $\alpha$  antibody or antigen binding portion thereof is effective in treating the disorder, comprising administering to the human subject subcutaneously a composition containing an anti-TNF $\alpha$  antibody or an antigen binding portion thereof and methotrexate such that the disorder is treated.

35 29. The method of claim 28, wherein methotrexate is administered together with the administration of an anti-TNF $\alpha$  antibody or antigen binding portion thereof.

30 30. The method of claim 28, wherein methotrexate is administered prior to the administration of an anti-TNF $\alpha$  antibody or antigen binding portion thereof.

35 31. The method of claim 28, wherein methotrexate is administered after the administration of an anti-TNF $\alpha$  antibody or antigen binding portion thereof.

32. The method of claim 28, wherein said anti-TNF $\alpha$  antibody is a human anti-TNF $\alpha$  antibody or antigen binding portion thereof.

5 33. The method of claim 28, wherein said human antibody, or an antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_d$  of  $1 \times 10^{-8}$  M or less and a  $K_{off}$  rate constant of  $1 \times 10^{-3} s^{-1}$  or less, both determined by surface plasmon resonance, and neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC<sub>50</sub> of  $1 \times 10^{-7}$  M or less.

10 34. The method of claim 28, wherein said human antibody, or antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  rate constant of  $5 \times 10^{-4} s^{-1}$  or less.

15 35. The method of claim 28, wherein said human antibody, or antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  rate constant of  $1 \times 10^{-4} s^{-1}$  or less.

20 36. The method of claim 28, wherein said human antibody, or antigen-binding portion thereof, neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC<sub>50</sub> of  $1 \times 10^{-8}$  M or less.

25 37. The method of claim 28, wherein said human antibody, or antigen-binding portion thereof, neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC<sub>50</sub> of  $1 \times 10^{-9}$  M or less.

38. The method of claim 28, wherein said human antibody, or antigen-binding portion thereof, neutralizes human TNF $\alpha$  cytotoxicity in a standard *in vitro* L929 assay with an IC<sub>50</sub> of  $1 \times 10^{-10}$  M or less.

30 39. The method of claim 28, wherein said human antibody, or antigen-binding portion thereof, is a recombinant antibody, or antigen-binding portion thereof.

40. The method of claim 28, wherein said human antibody, or antigen-binding portion thereof, inhibits human TNF $\alpha$ -induced expression of ELAM-1 on human umbilical 35 vein endothelial cells.

41. A method for inhibiting human TNF $\alpha$  activity in a human subject suffering from a disorder in which TNF $\alpha$  activity is detrimental, comprising administering to the

human subject methotrexate and subcutaneously, a human anti-TNF $\alpha$  antibody wherein said human antibody, or antigen-binding portion thereof, has the following characteristics:

- a) dissociates from human TNF $\alpha$  with a  $K_{off}$  rate constant of  $1 \times 10^{-3} \text{ s}^{-1}$  or less, as determined by surface plasmon resonance;
- 5 b) has a light chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8 or by one to five conservative amino acid substitutions at positions 1, 3, 4, 6, 7, 8 and/or 9;
- 10 c) has a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 or by one to five conservative amino acid substitutions at positions 2, 3, 4, 5, 6, 8, 9, 10, 11 and/or 12.

15 42. The method of claim 41, wherein said human antibody, or an antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  rate constant of  $5 \times 10^{-4} \text{ s}^{-1}$  or less.

20 43. The method of claim 41, wherein said human antibody, or an antigen-binding portion thereof, dissociates from human TNF $\alpha$  with a  $K_{off}$  rate constant of  $1 \times 10^{-4} \text{ s}^{-1}$  or less.

25 44. A method for inhibiting human TNF $\alpha$  activity in a human subject suffering from a disorder in which TNF $\alpha$  activity is detrimental, comprising administering to the human subject methotrexate and subcutaneously a human anti-TNF $\alpha$  antibody, wherein said human antibody, or an antigen-binding portion thereof, has a light chain variable region (LCVR) having a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8, and has a heavy chain variable region (HCVR) having a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11.

30 45. The method of claim 44, wherein the LCVR of said human antibody, or an antigen-binding portion thereof, further has a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 5 and the HCVR further has a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 6.

46. The method of claim 44, wherein the LCVR of said human antibody, or an antigen-binding portion thereof, further has CDR1 domain comprising the amino acid

sequence of SEQ ID NO: 7 and the HCVR has a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 8.

47. A method for inhibiting human TNF $\alpha$  activity in a human subject suffering from a disorder in which TNF $\alpha$  activity is detrimental, comprising administering to the human subject methotrexate and subcutaneously a human anti-TNF $\alpha$  antibody wherein said human antibody, or an antigen binding portion thereof, has a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 1 and a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 2.

10

48. The method of claim 47, wherein said human antibody has an IgG1 heavy chain constant region.

15

49. The method of claim 47, wherein said human antibody has an IgG4 heavy chain constant region.

50. The method of claim 47, wherein said human antibody is a Fab fragment.

20

51. The method of claim 47, wherein said human antibody is a single chain Fv fragment.

25

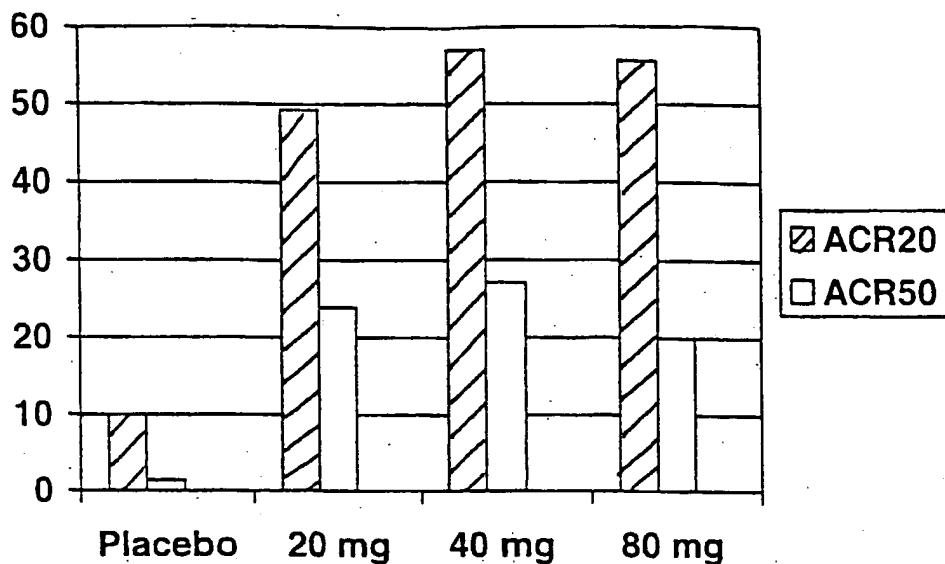
52. A method for inhibiting human TNF $\alpha$  activity in a human subject suffering from a disorder in which TNF $\alpha$  activity is detrimental, comprising administering to the human subject methotrexate and subcutaneously a human anti-TNF $\alpha$  antibody, or an antigen-binding portion thereof, which has a light chain variable region (LCVR) having a CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26 or has a heavy chain variable region (HCVR) having a CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34.

35

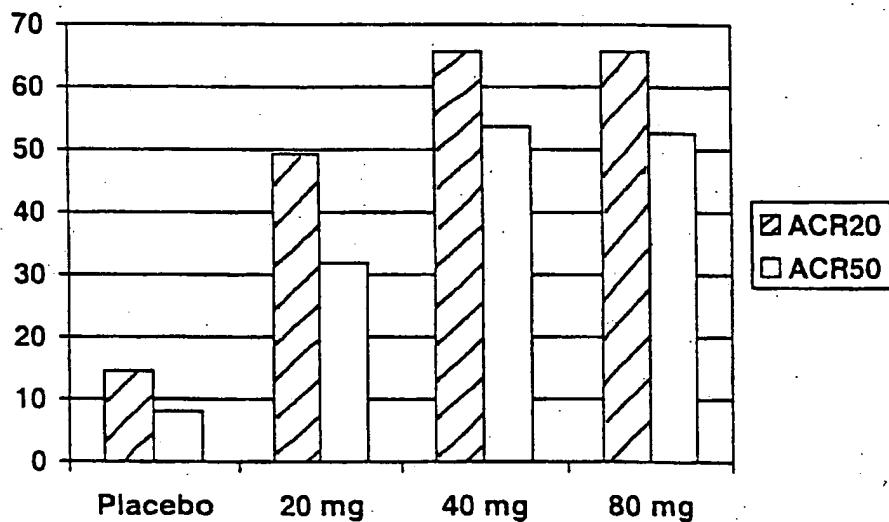
53. A method for inhibiting human TNF $\alpha$  activity in a human subject suffering from a disorder in which TNF $\alpha$  activity is detrimental, comprising administering to the human subject methotrexate and subcutaneously a human anti-TNF $\alpha$  antibody wherein said human antibody is the antibody D2E7 or an antigen-binding portion thereof.

54. A kit comprising a formulation comprising:
- a) a pharmaceutical composition comprising an anti-TNF $\alpha$  antibody or an antigen binding portion thereof, methotrexate and a pharmaceutically acceptable carrier; and
  - b) instructions for subcutaneous dosing to a subject of the pharmaceutical composition for the treatment of a disorder in which TNF $\alpha$  activity is detrimental.
- 5 55. The kit of claim 54, wherein said antibody comprises the antibody, or antigen-binding portion thereof, as set forth in any one of claims 32-51.
- 10 56. A preloaded syringe containing a pharmaceutical composition comprising an anti-TNF $\alpha$  antibody, methotrexate, and a pharmaceutically acceptable carrier.
57. The syringe of claim 56, wherein said antibody comprises the antibody or antigen-binding portion thereof, as set forth in any one of claims 32-52.
- 15 58. The method of claims 1 or 28, wherein the disorder is sepsis.
59. The method of claims 1 or 28, wherein the antibody is administered to the human subject together with the cytokine interleukin-6 (IL-6) or is administered to a human subject with a serum or plasma concentration of IL-6 above 500 pg/ml.
- 20 60. The method of claims 1 or 28, wherein the disorder is an autoimmune disease.
61. The method of claim 60, wherein said autoimmune disease is selected from the group consisting of rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis and gouty arthritis.
- 25 62. The method of claim 61, wherein said autoimmune disease is rheumatoid arthritis.
- 30 63. The method of claim 60, wherein said autoimmune disease is selected from the group consisting of an allergy, multiple sclerosis, autoimmune diabetes, autoimmune uveitis and nephrotic syndrome.
- 35 64. The method of claims 1 or 28, wherein the disorder is an infectious disease.
65. The method of claims 1 or 28, wherein the disorder is transplant rejection or graft-versus-host disease.

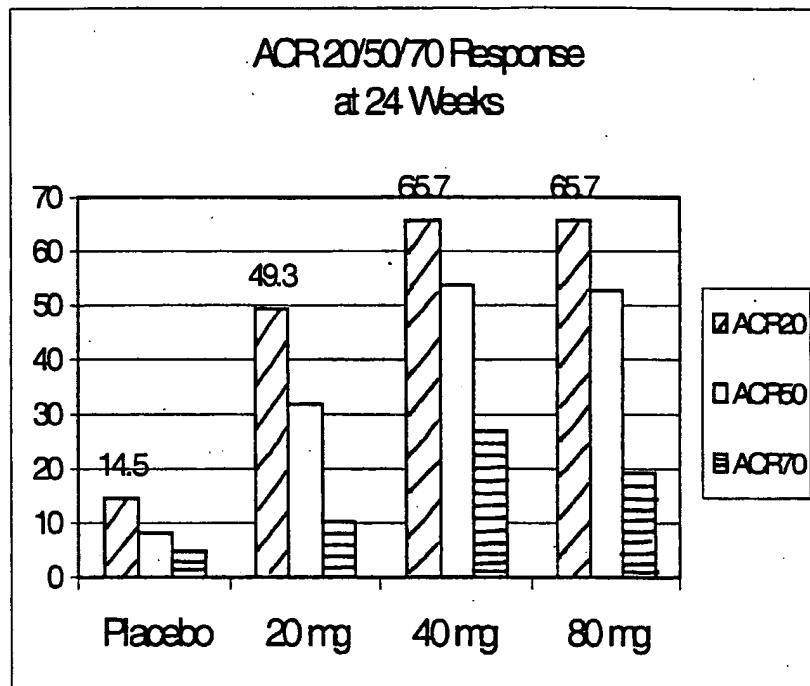
66. The method of claims 1 or 28, wherein the disorder is a malignancy.
67. The method of claims 1 or 28, wherein the disorder is a pulmonary disorder.
- 5 68. The method of claims 1 or 28, wherein the disorder is an intestinal disorder.
69. The method of claims 1 or 28, wherein the disorder is a cardiac disorder.
- 10 70. The method of claims 1 or 28, wherein the disorder is selected from the group consisting of inflammatory bone disorders, bone resorption disease, alcoholic hepatitis, viral hepatitis, coagulation disturbances, burns, reperfusion injury, keloid formation, scar tissue formation and pyrexia.
- 15 71. A kit comprising:
  - a) a pharmaceutical composition comprising an anti-TNF $\alpha$  antibody or an antigen binding portion thereof and a pharmaceutically acceptable carrier;
  - b) a pharmaceutical composition comprising methotrexate and a pharmaceutically acceptable carrier; and
  - c) instructions for subcutaneous dosing to a subject of the anti-TNF $\alpha$  antibody pharmaceutical composition and dosing of the methotrexate pharmaceutical composition before, simultaneously or after the dosing of the anti-TNF $\alpha$  antibody pharmaceutical composition.
- 20 72. A kit according to claim 71, wherein the anti-TNF $\alpha$  antibody or an antigen binding portion thereof is D2E7 or an antigen binding portion thereof.
- 25



**Study DE007**  
**Dosing every week**

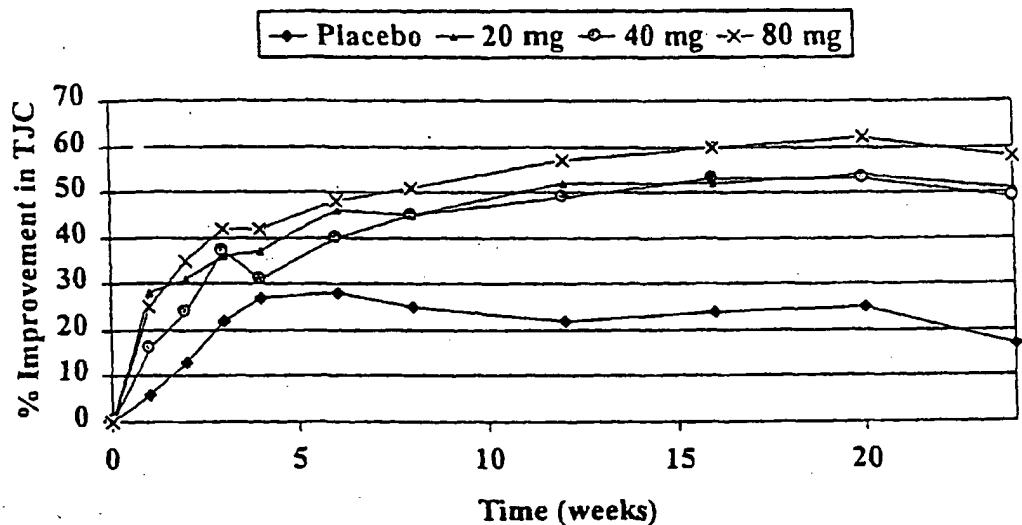


**Study DE009**  
**Dosing every other week**

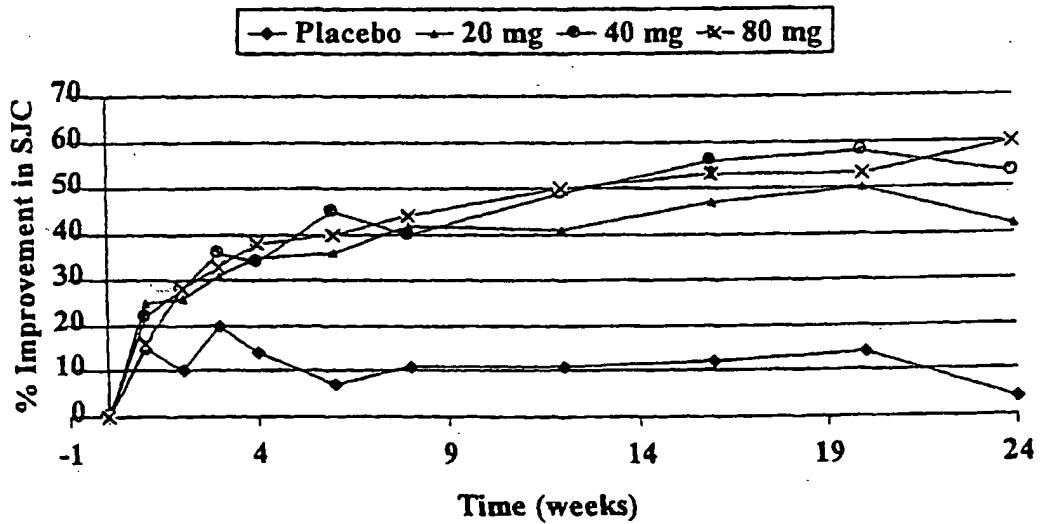
**FIGURE 2**

**A**

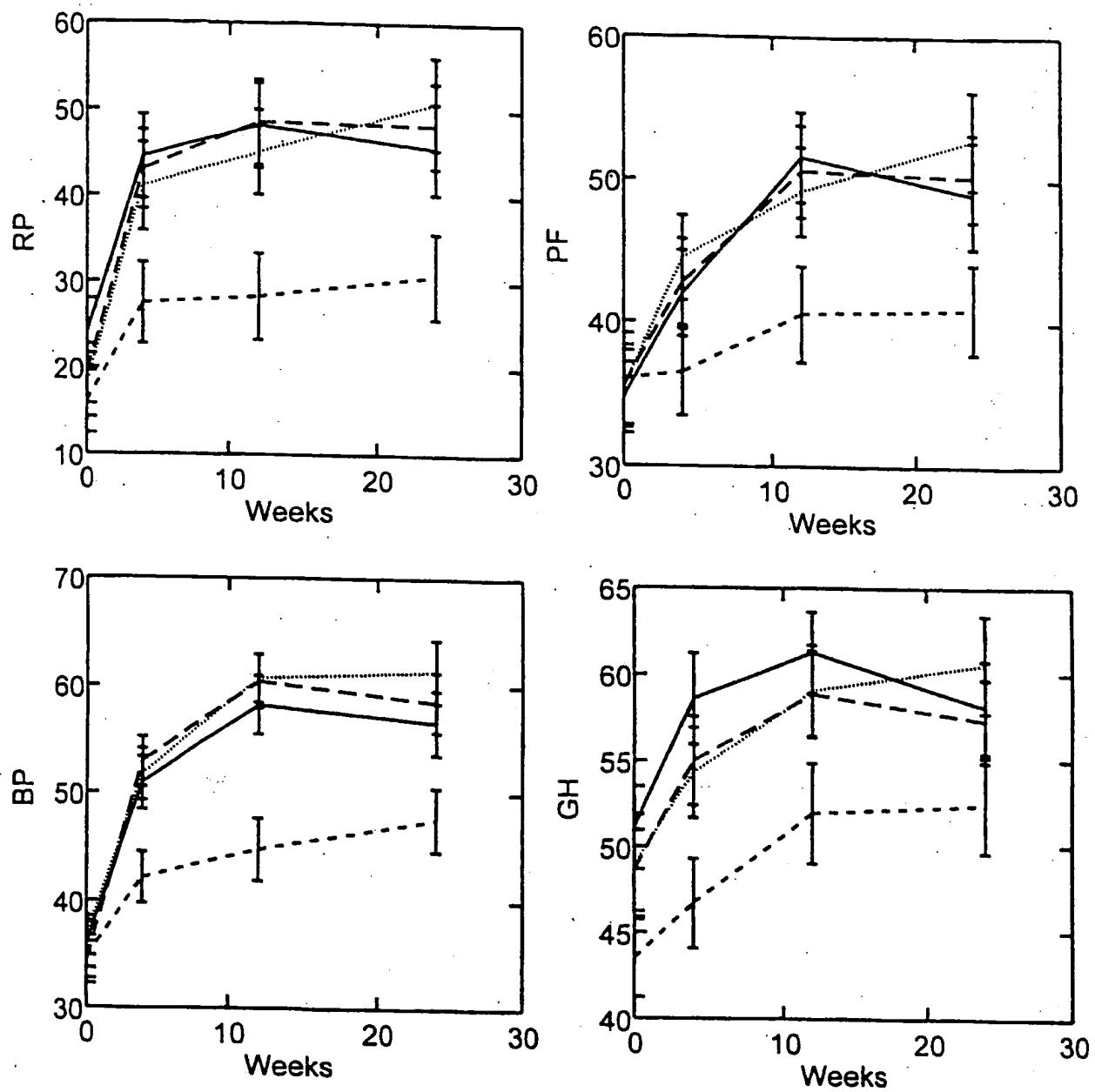
## Course of Tender Joint Count over 24 Weeks

**B**

## Course of Swollen Joint Count over 24 Weeks

**FIGURE 3**

# SF-36 SCALE



**FIGURE 4**

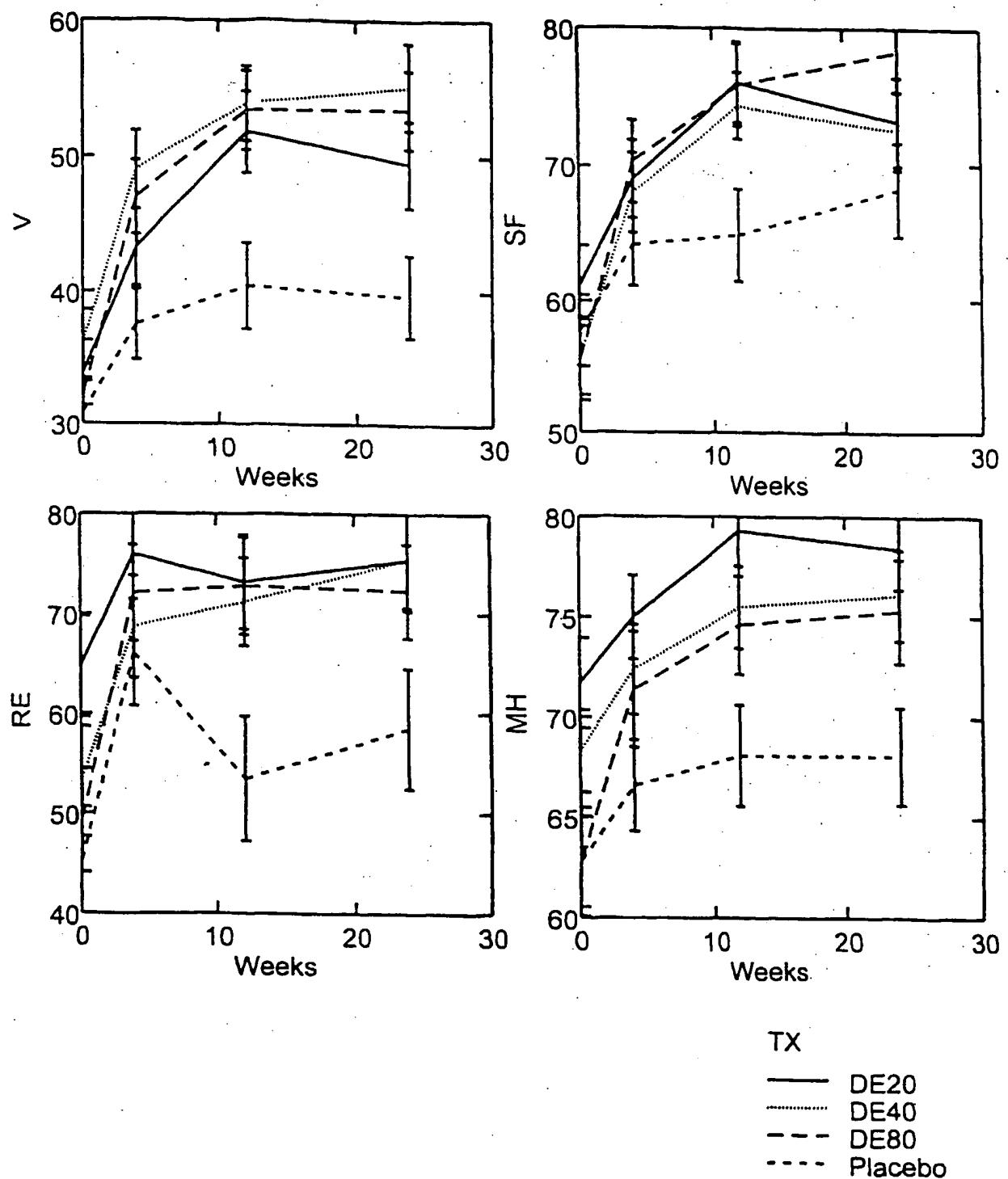


FIGURE 4

Percentage of ACR20 responders following a single i.v. injection of D2E7 and/or placebo (DE010)

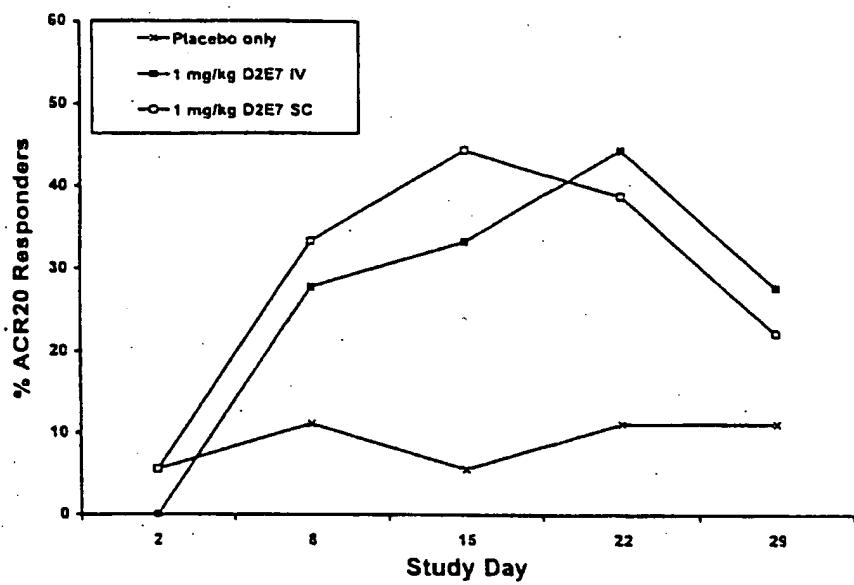


FIGURE 5

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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**PCT/US2002/017790**

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(25) Filing Language: **English**

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(71) Applicant (*for all designated States except US*): **ABBOTT Biotechnology Ltd [GB/GB]; Clarendon House, 2 Church Street, HM 11 Hamilton (BM).**

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **KEMPENI, Joachim [DE/DE]; 75 Gimmeldinger Street, 67433 Neustadt (DE). WEISS, Roberta [US/US]; 936 Clover Hill Road, Wynnewood, PA 19096 (US). FISCHKOFF, Steven, A. [US/US]; 5 Canoe Brook Road, Short Hills, NJ 07078 (US).**

(74) Agent: **DECONTI, Jr., Giulio, A.; Lehive & Cockfield, LLP, 28 State Street, Boston, MA 02109-1784 (US).**

Declaration under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for all designations*

Published:

- *with international search report*

(88) Date of publication of the international search report:  
**12 February 2004**

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 2002/100330 A3

(54) Title: **METHODS OF ADMINISTERING ANTI-TNF $\alpha$  ANTIBODIES**

(57) Abstract: Methods of treating disorders in which TNF $\alpha$  activity is detrimental via biweekly, subcutaneous administration of human antibodies, preferably recombinant human antibodies, that specifically bind to human tumor necrosis factor  $\alpha$ (hTNF $\alpha$ ) are disclosed. The antibody may be administered with or without methotrexate. These antibodies have high affinity for hTNF $\alpha$  (e.g.,  $K_d = 10^{-8}$  M or less), a slow off rate for hTNF $\alpha$  dissociation (e.g.,  $K_{off} = 10^{-3}$  sec $^{-1}$  or less) and neutralize hTNF $\alpha$  activity *in vitro* and *in vivo*. An antibody of the invention can be a full-length antibody or an antigen-binding portion thereof. Kits containing a pharmaceutical composition and instructions for dosing, and preloaded syringes containing pharmaceutical compositions are also encompassed by the invention.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/17790

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 39/395; C07K 16/00  
 US CL : 424/130.1, 133.1, 156.1; 530/387.1, 387.3, 388.15, 388.8, 388.85

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
 U.S. : 424/130.1, 133.1, 156.1; 530/387.1, 387.3, 388.15, 388.8, 388.85

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
 Please See Continuation Sheet

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,090,382 A (SALFIELD ET AL) 18 July 2000 (18/07/00), see entire document, especially column 21, line 19-24, column 22, lines 40-61, and SEQ ID NOs.	1-10, 17-43, and 47-72
Y	US 6,214,870 (MCCLURE ET AL) 10 April 2001 (10/04/01), see entire document, especially column 11, lines 63-65.	1-10, 17-43, and 47-72

<input type="checkbox"/>	Further documents are listed in the continuation of Box C.	<input type="checkbox"/>	See patent family annex.
*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier application or patent published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search	24 February 2003 (24.02.2003)	Date of mailing of the international search report	06 AUG 2003
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Larry R. Helms Telephone No. (703) 308-0196	<i>Susan C. Wolstorf</i>	

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/17790

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claim Nos.: 11-16, 44-46 and part of claims 22, 25, 27, 52, 55, and 57  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
The above indicated claims require the search of SEQ ID NO 4 which has not been disclosed in the application as filed.
  
3.  Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

PCT/US02/17790

**INTERNATIONAL SEARCH REPORT**

**Continuation of B. FIELDS SEARCHED Item 3:**  
CAPLUS, MEDLINE, BIOSIS, WEST, CANCERLIT  
search terms: antibody, TNF-alpha, treatment, biweekly